The mechanisms and timing of mineralization of fossil phosphatized soft tissues

Thesis

The mechanisms and timing of mineralization of fossil phosphatized soft tissues

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Abstract

Fossil phosphatized soft tissues offer palaeontologists a unique opportunity to examine the biology and physiology of extinct organisms at the cellular and even macromolecular level. All phosphatized soft tissues are preserved by one or more of three preservational styles. These are: 1) phosphatized microbial infestations, 2) non-microbial (i.e. inorganic) phosphatic coatings, and 3) inorganic replacements. This suggests that three different (but related) processes are involved in the phosphatization of soft tissues. Each of these processes preserves the tissues at a predictable resolution; the most detailed preservation being afforded by inorganic replacements. In general, the soft tissues of closely related organisms have similar preservational styles. This reflects similarities in the biochemistry and taphonomy of closely related taxa.

In the *majority* of cases of soft tissue phosphatization, the most important source of phosphorus appears to have been external to the organism undergoing mineralization. An accessible source of phosphorus is, however, not the only variable dictating mineralization; phosphatization is extremely taxon-, tissue-, and biomolecule-specific. Of particular importance is: a) the concentration of phosphorus in the organism's soft tissues; b) the tissue's proximity to the source of phosphorus; c) the rate of tissue decay relative to the onset of mineralization; and, d) the pH and chemical composition of the decay-induced microenvironment surrounding the carcass. Certain groups of organisms (e.g. crustaceans, squid, and fish) appear to be somewhat 'preconditioned' for phosphatization.

All fossil phosphatized soft tissues exhibit evidence of decay. Taphonomic experiments suggest the period between death and phosphatization to have been as little as 55 hours. In the case of microbial infestation, decay would have permitted the microbes to gain access to the carcass, and to release organically-bound phosphorus from its tissues. In inorganic phosphatization, decay stimulates mineralization by: a) degrading membranes and thus accelerating the rate at which dissolved phosphorus and calcium may invade the tissues; b) creating new "reactive" organic substrates on which apatite may precipitate; c) destroying intracellular nucleation inhibitors; and, d) creating a favourable chemical microenvironment for the precipitation of apatite. Inorganic postmortem phosphatization may therefore be considered to be an "end-member" of pathological biomineralization.

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1.1 INTRODUCTION

Phosphatized soft tissues display some of the most exceptional, three-dimensional ultrastructural details of any fossils (e.g. see Martill, 1988; Schultze, 1989). They are more common (see Müller, 1985, p67), and temporally (Cambrian to Recent) and palaeoenvironmentally more widespread (see Allison and Briggs, 1991a, Table IV pp44-47) than generally believed (table 1.1). Indeed, in certain deposits (e.g. the Alum Shale Formation, Sweden, see Müller, 1985) phosphatized soft tissues may be relatively abundant.

Perhaps the most celebrated contribution of phosphatized soft tissues to a specific palaeontological problem was the clarification of the conodont animal's taxonomic affinities (Briggs *et al.*, 1983). However, phosphatized soft tissues have made a number of other important contributions. Müller (1979) and subsequently Müller and Wallosek (1985a) described three-dimensionally preserved arthropods from the Upper Cambrian Alum Shale Formation (Orsten) of Sweden. These provided valuable insights into the early evolution, ecology, and ontogeny of Crustacea, and lent support to Whittington's (1979) contention of an arthropod dominated Cambrian fauna. Similarly, the pterosaur wing membrane described by Martill and Unwin (1989) from the Romualdo Member of NE Brazil is of immense palaeobiological importance. This was preserved in such exceptional detail that it gave an opportunity to comment on the flight mechanisms and thermoregulation of pterosaurs based for the first time on unequivocal fossil evidence. In the same deposit, phosphatized soft tissues have also contributed towards an understanding of predator/prey relationships (Wilby and Martill, 1992), parasitism (Cressey and Patterson, 1973), scavenging (Bate, 1971) and even sexuality (Bate, 1973).

Thus, phosphatized soft tissues are of particular palaeobiological importance.

NAME	LOCATION	AGE	REFERENCE
Potterne Midden	Wiltshire, UK	Recent	Piearce et al., 1990
Eckfeld Maarlake	SW Eifel, Germany	Eocene	Micklich and Wuttke, 1988
"Kysylkum"	USSR	Cretaceous	Martinson et al., 1986
Niobrara Chalk Formation	USA	Cretaceous	Stewart, 1990
Haqel Basin	Lebanon	Cenomanian, Upr Cretaceous	Hückel, 1970
Romualdo Member, Santana Fm	Chapada do Araripe, Ceara, NE Brazil	Aptian/Albian, Lwr Cretaceous	Martill, 1990b
Crato Formation	Chapada do Araripe, Ceara, NE Brazil	Aptian/Albian, Lwr Cretaceous	Grimaldi, 1990
"Volga River"	Saratov district, USSR	Upr Jurassic	Dzik, 1978
Portland Roach, Winspit Member	Isle of Portland, UK	Portlandian, Upr Jurassic	Whyte, 1991
Solnhofen Lithigraphic Limst	West Germany	Tithonian, Upr Jurassic	Reis, 1898
Cordillera de Domeyko	N. Chile	Oxfordian, Upr Jurassic	Schultze, 1989
Oxford Clay Formation	Christian Malford, Witshire, UK	Callovian, Mid Jurassic	Donovan and Crane, 1992
Lombardische Kieselkalk Fm	Osteno, N Italy	Sinemurian, Lwr Jurassic	Pinna, 1985
Lwr Lias	Leicestershire and Somerset, UK	Hettangian, Lwr Jurassic	Martin et al., 1986
Barents-Øya Formation	Spitsbergen	Mid Trias	Weitschat, 1986
Sticky Keep Formation	Spitsbergen	Lwr Triassic	Weitschat, 1983a and b
Lake Odernheim, Saar-Nahe Basin	SW Gemany	Lwr Permian	Willems and Wuttke, 1987
Granton shrimp Bed	Granton, Edinburgh, Scotland	Mississippian, Lwr Carboniferous	Briggs et al., 1991
Gullane shrimp Bed	E Scotland	Mississippian, Lwr Carboniferous	Cater et al., 1989
Glencartholm Volcanic Beds	Eskdale, Dumfriesshire, S Scotland	Mississippian, Lwr Carboniferous	Traquair, 1884
Manse Burn Formation	Glasgow, Scotland	Mississippian, Lwr Carboniferous	Clark, 1989
"Cephalopod Limestone"	Carnic Alps, Austria	Upr Devonian	Müller, 1982
Cleveland Shale	Ohio, USA	Upr Devonian	Dean, 1909
Unknown	Öland, Sweden	Lwr Ordovician	Andres, 1989
Alum Shale Fm, or "Orsten fauna"	Sweden and Poland	Upr Cambrian	Müller, 1985
Comley and Rushton Fauna	Shropshire, UK	Lwr Cambrian	Hinz, 1987

Table 1.1: The stratigraphical position of largerstätten containing a biota with phosphatized soft tissues

1.2 AIMS OF THIS THESIS

Despite the enormous palaeobiological importance of phosphatized soft tissues, surprisingly few detailed investigations have focused on the process(es) responsible for their mineralization. Speculation regarding the genesis of crystal microfabrics replacing phosphatized soft tissues has been rife, but has remained largely unresolved (e.g. see Allison, 1988d). Furthermore, established models of postmortem mineralization have recently been questioned (see Maisey's 1991, pp79-84 review of Martill 1988) and there is little agreement over the timing of the mineralizing event (e.g. Schultze, 1989, and Martill and Harper, 1990). This has left research in a state of flux.

Clearly, a comprehensive synthesis of current knowledge is well overdue. This thesis tackles many of the outstanding problems of soft tissue phosphatization. Emphasis is placed on the mechanisms of mineralization at the biomolecular and cellular levels. The investigation focuses principally on phosphatized soft tissues from the Romualdo Member of the Araripe Basin, NE Brazil. Martill (1990a, b) has demonstrated that this material is exceptionally well preserved, and other workers (e.g. Bate, 1971; Cressey and Patterson, 1973; Martill and Unwin, 1989; Wilby and Martill, 1992) have proven phosphatized soft tissues to occur in a number of taxa. This permits the crystallite/tissue relationship(s) to be examined at the greatest possible resolution, and ensures that any taxonomic biases are identified.

I discuss five key areas of soft tissue phosphatization in the Romualdo Member:

1) The abundance, diversity, and lateral- and taxonomic-distribution of the phosphatized soft tissues in the Araripe Basin.
2) The characteristic preservational styles of the soft tissues, and the microstructure of the replacing crystal fabrics.
3) The timing of the mineralizing event relative to the death and decay of the organisms.
4) The source of the phosphorus.
5) The role of microenvironments and organic substrates in phosphatization.

With the possible exception of the Orsten Fauna where the distribution and abundance of phosphatized soft tissues is fairly well constrained (see Müller and Walossek, 1987), comprehensive studies of the phosphatized soft tissues of individual deposits have not been

performed. This thesis presents such a study. The ultimate goal is to achieve a more complete understanding of the sedimentological, environmental, and biological controls on postmortem phosphatization.

1.3 THESIS LAYOUT

This thesis is divided into nine chapters:

Chapter 2 reviews the sedimentology of the Araripe Basin and includes a revision of its lithostratigraphy. A palaeoenvironmental analysis of the lithology bearing the phosphatized soft tissues (the Romualdo Member) is presented based on its lithological, faunal, taphonomic and diagenetic characteristics.

Chapter 3 gives a detailed description of the microfabrics replacing the soft tissues of the Romualdo Member biota. Interpretations regarding the mechanisms of each microfabric's genesis are based principally on comparisons with published photomicrographs of material from phosphorites and apatites precipitated *in vitro*.

Chapter 4 examines the phosphatized soft tissues of the Romualdo Member and describes their preservation. This 'atlas of soft tissues' provides the data base from which many of the conclusions of later chapters are drawn, and demonstrates the diversity of soft tissues which may be phosphatized in a single deposit.

Chapter 5 examines the taxonomic distribution of phosphatized soft tissues, and investigates taxon-related trends in preservational styles. Two classification schemes are introduced: one based on the characteristic preservational style of each biota, and the other on the fidelity with which the tissues are replaced.

Chapter 6 describes the successive stages in the decomposition of striated fish muscle, and compares these with the state of preservation of fossil muscle from a number of deposits. The precise replication in actualistic experiments of taphonomic structures which are preserved in the fossil muscle gives a semi-quantitative estimate of the timing of phosphatization and of the palaeosalinity of the Romualdo Member.

Chapter 7 discusses the likely/possible source(s) of phosphorus in a number of deposits containing phosphatized soft tissues. Particular attention is paid to the palaeoenvironmental characteristics of each deposit, and the quantity of phosphorus available within the

organisms themselves. A model (based on data from regions of modern phosphogenesis) is given for the Romualdo Member, and the most favourable environmental conditions for phosphatization are discussed.

Chapter 8 presents a model of postmortem phosphatization for the Romualdo Member. This is based largely on data given in the earlier chapters, and on current theories of biomineralization. The importance of chemical microenvironments, nucleation inhibitors, organic templates, rates of decay, and the concentration of phosphorus within the tissues are discussed.

Each chapter ends with a summary of the principal conclusions reached. These are re-iterated in my concluding chapter, and avenues for future research are suggested.

1.4 MATERIALS USED IN THIS STUDY

Both fossil and living material has been utilized in this study. The majority of the fossil material required for semi-destructive analysis was collected from the Romualdo Member of the Chapada do Araripe, NE Brazil. Additional material from the same deposit was acquired from 'Rock Art' (contact Terry Manning, 4-6 Gipsy Lane, Leicester) or was kindly provided by the Departamento Nacional da Produção Mineral (DNPM), Crato, Brazil. Most specimens from the Portland Roach were collected on a short field excursion in 1992 to the Isle of Portland, Dorset, although some were kindly donated by Dr. Whyte (University of Sheffield). Specimens of *Tealliocaris* sp. and *Waterstonella* sp. from the Gullane and Granton shrimp beds respectively, were gratefully accepted from Dr. Clarkson (University of Edinburgh). Dr. Piearce (University of Lancaster) was kind enough to donate a few phosphatized earthworm eggs from a Wiltshire midden. All of the above material is identified in the following text by the prefix "PRW".

Material from a number of other deposits lodged in various institutions, was also sampled or is referred to in the text. Specimens from the British Natural History Museum are identified in the text by the prefix "BMNH"; those from the New Walk Museum, Leicester by "LEICS"; those lodged with Leicester University by "LEIUG"; material from the American Museum of Natural History by "AMNH"; that located in the DNPM at Crato,

Ceara, NE Brazil by "CPCR"; and material from the National Museum of Scotland by the prefix "NMS".

Comments relating to phosphatized soft tissues from deposits which I was unable to sample are based entirely on published figures. These I take to be representative samples of these deposits as a whole. Reference to published figures is in every case clearly signalled.

Live material came from two sources depending on the requirement. Material for gross taphonomic studies was purchased frozen from large supermarkets with a high turnover of goods to ensure freshness. Specimens required for histological examination were received live (after overnight travel) from the University Marine Biological Station, Millport, Cumbrae, Scotland.

Appendix 1 lists all the biological and fossil material used in this study, giving their identification number, method of preparation and collection locality.

1.5 METHODOLOGY

The use of live and fossil material necessitates the application of a wide range of techniques, some of which may be new to palaeontologists. Protocols for Critical Point Drying (CPD), Energy Dispersive Analysis of X-rays (EDAX), and the embedding of Recent and fossil tissues in resins are given in Appendix 3.

1.5.1 NOTE ON SCANNING (SEM) AND TRANSMISSION (TEM) ELECTRON MICROSCOPE USE

Extensive use has been made of electron microscopes. Fossil and CPD material for examination on the SEM was attached to Cambridge stubs with silver dag (BioRad A1208) and coated with gold (intermittently rotating the specimen) for 10 minutes. Long coating periods were necessary, particularly for the fossil material, due to its friable nature and considerable topography.

All fossil material examined in TEM was with a JEOL 2000FX at 200 Kv. 'Live' material was examined on a JEOL 100S. All photomicrographs are located in volume 2 of this thesis and are referred to in the text as "fig.". Graphical and explanatory figures which are located in this volume are referred to in the text as "text fig.".

1.6 PHOSPHATIZED SOFT TISSUES: PREVIOUS INVESTIGATIONS

Recently the use of various geochemical hardware and high resolution SEMs has stimulated a renewed interest in deposits containing exceptional fossil material (or Conservation Lagerstätten, *sensu* Seilacher *et al.*, 1985). This has led to the discovery of a number of new deposits containing phosphatized soft tissues, and to a desire to understand more fully their sedimentological, geochemical and taphonomic characteristics. This ethos has encouraged the development of models of soft tissue phosphatization in which elements of biomineralogy, bacteriology and geochemistry have been integrated.

In recent years research has focused on three principle areas of controversy: the source of phosphorus; the mechanism(s) of phosphatization; and the timing (relative to death) of mineralization. Advances in these three areas are reviewed separately below in roughly historical order:

1.6.1 SOURCE OF PHOSPHORUS

Reis (1893, 1895, 1898) was one of the first palaeontologists to seriously consider the source of phosphorus necessary for the phosphatization of soft tissues. He (Reis, op. cit.) examined the phosphatized muscle of fish and ichthyosaurs from the Solnhofen Lithographic Limestone (Germany) and concluded the phosphorus to have been derived from an internal source. Originally, he (Reis 1893, 1895) invoked a correlation with carnivorous behaviour; the dissolved phosphorus diffusing to the site of mineralization from bone-rich stomach contents. However, the preservation of soft tissues in pycnodonts with globident dentition (i.e. molluscivores) later prompted him (according to Dean, 1902, p275) to propose the phosphorus to have been derived from the organisms' own muscle and blood.

Dean (1902, 1909), based on the phosphatized muscle, kidneys and stomach contents of sharks from the Cleveland Shale (Devonian) of Ohio, postulated the phosphorus to be derived from the sediment. This he suggested, was implied by the preservation of thin, peripheral crusts of phosphatized soft tissues on only the lower surface of each shark.

Bate (1972) similarly entertained an external source. He (Bate, 1971, 1972, 1973) described exceptionally well preserved specimens of a new species of cypridid ostracode from the Romualdo Member of NE Brazil, which had apparently been feeding on associated carcasses of the fish - *Cladocyclus gardneri* Agassiz. The apparent lack of decay, and the occurrence of appendages in life position (including one specimen with the copulatory organ in a partially extended position), led Bate (1971) to propose the ostracodes to have been asphyxiated and buried within the decaying tissues of the fish. Further decomposition of the fish provided the necessary flux of phosphorus for the mineralization of the entombed ostracodes.

Martill (1988, 1989a) described the phosphatized soft tissues of fish from the Romualdo Member and postulated three alternative sources for the phosphorus. In the first, he (Martill, 1988, p11) proposed the phosphorus to have come from the sediment, the overlying water column, and from phosphate-rich biomolecules within the carcasses themselves. In the second, he (Martill, 1989a, fig. 2) envisaged the bottom waters of the basin to have become progressively enriched in dissolved phosphorus through the microbial reworking of fish killed in mass mortality events. The third, and somewhat unorthodox model offered by Martill (1989a, fig. 3) - "the pterosaur crap model" - postulated phosphate rich guano to have been washed down into the water column from large pterosaur nesting grounds at the periphery of the basin!

Wilby (1993) identified clear gradients in the density of phosphatization within individual fish of the Romualdo Member. At the periphery, just beneath the dermis, the skeletal muscle was densely mineralized, whereas at greater depths within the fish, the soft tissues were much less extensively mineralized. This, he (Wilby, op. cit.) proposed, is consistent with phosphate ions having diffused into the carcass from an external source.

Müller (1979, 1985) noted phosphatized arthropods in the Alum Shale Formation of Sweden to have a very similar style of preservation to the ostracodes previously described by Bate (1971, 1972, 1973) from the Romualdo Member (see above). However, in the absence of any obvious, large, associated source of phosphate-rich organic matter (Bate's

dead fish), he (Müller, op. cit.) concluded the phosphorus to have been derived from: 1) granite hinterlands; and/or 2) the soft- and mineralized tissues of the associated phosphatic shelly fauna.

The viability of organic material as a source of phosphorus for the mineralization of microbes was experimentally tested by Lucas and Prévôt (1984), Prévôt *et al.* (1989), and Hirschler *et al.* (1990a, 1990b). These authors demonstrated that certain bacteria under normal marine conditions can synthesize apatite when provided with an organic source of phosphorus (RNA) and a dissolved or crystalline source of calcium. Subsequently, an organic source of phosphorus has been invoked for the phosphatized soft tissues of a number of fossil deposits including the Granton and Gullane shrimp beds (Cater *et al.*, 1989; Briggs *et al.*, 1991) and the Romualdo Member (Martill, 1988, 1989a). Indeed, the intimate relationship between microbes and soft tissues in many deposits has led several workers to propose the substrate tissue and/or the decomposing organisms themselves to be the ultimate source of phosphate ions. Such a source has been invoked for soft tissues from Eckfeld Maarlake (Micklich and Wuttke, 1988), the Romualdo Member (Martill, 1988), the Solnhofen Lithographic Limestone (Mehl, 1990), and Lake Odernheim (Willems and Wuttke, 1987), and has recently been experimentally confirmed by Briggs and Kear (1993a). Clearly an external organic source of phosphorus is not a prerequisite for the postmortem phosphatization of *all* soft tissues.

Allison (1988a) has proposed the concentration of dissolved phosphorus in the pore waters of sediments may be secondarily enriched by the release of phosphate ions (at the redox boundary) which were adsorbed onto ferric ions (see Benmore *et. al.* 1983). This process was considered by Cater *et al.* (1989) and Briggs *et al.* (1991) to have been active in the shrimp-bearing Lower Carboniferous sediments of northern Britain.

1.6.2 PALAEOENVIRONMENTAL CONTROLS AND MECHANISMS OF PHOSPHATIZATION

Müller (1985) was the first to seriously consider the mechanisms of soft tissue phosphatization. He proposed the preferential phosphatization of arthropods in the Alum Shale Formation to reflect selective mineralization of chitinous material; the arthropod exoskeletons acting as templates for the precipitation of apatite. Distortion of the inner lamellae of many ostracodes led Müller (1985) to further suggest the precipitation of the phosphate (probably an initially amorphous phase) to have been triggered by minor fluctuations in the salinity of the water column.

Allison (1988a) reviewed the processes of soft tissue fossilization and discussed two modes of preservation relevant to phosphatized soft tissues. In the first ("permineralization"), the soft tissues are preserved in exceptional three-dimensional detail by the extremely rapid (relative to decay) precipitation of phosphates; whilst in the second ("mineral coats"), apatite nucleates only onto the tissue's outer surface(s). Allison (1988a) conceded that very little was known of the mechanism(s) involved in the latter mode of preservation, but contrary to Müller (1985), proposed it to be bacterially mediated.

Allison emphasized several other points including the importance of 1) rapid phosphatization in preventing decay-induced information loss (Allison 1988c); 2) phosphatic particles such as vertebrate and arthropod fragments as precipitation nuclei (Allison 1988b); and, 3) microenvironments produced by decaying carcasses in stimulating the precipitation of phosphates (Allison 1988a). He (Allison, 1988a, pp338-340) further proposed the phosphatization of soft tissues to be favoured by a high sedimentary organic content and a low rate of burial.

Allison (1988d) also performed a re-examination of the phosphatized soft tissues of teuthoids from the Lower Oxford Clay (Callovian, Upper Jurassic) of Wiltshire. He suggested three alternative mechanisms for their preservation, but was unable to establish which had actually been responsible. These are:

1) "decay-induced alkalinity" - the decomposing organism creates a specific microenvironment favourable to the precipitation of apatite.

2) "bacterial fixation" - the tissues become infested with bacteria which are either replaced by apatite or, upon autolysis (the breakdown of the cell after death following the release of lysosomal enzymes) deposit a phosphatic residue on the host tissue.

3) "formation of intermediate organic/inorganic complexes" - the decay of the tissues liberates proteolipids (known to catalyse apatite precipitation, see Ennever *et al.*, 1981) which being concentrated in the carcasses promote the precipitation of phosphate only here. In this model, the proteolipids act as both the template and catalyst for phosphate nucleation.

Cater *et al.* (1989) examined Carboniferous shrimp-bearing lithologies in northern Britain, and proposed a combination of palaeoenvironmental factors were involved in the phosphatization of their soft tissues. Of particular importance were the levels and timing of organic input into the sediment, grain size and rate of sedimentation, position of the redox boundary, and salinity.

Martill (1989b) named several other criteria which he considered to be important pre-requisites for phosphatization. These are: 1) quiet water conditions and a lack of scavengers; 2) rapid phosphatization relative to decay; 3) relatively rapid burial (contrary to Allison, 1988a); and, 4) the protective growth of pre-compactional concretions around the carcass. Martill (1988, 1990a) also consistently invoked bacterial sealing (*sensu* Seilacher *et al.*, 1985) as an important factor for the phosphatization of soft tissues in fish from the Romualdo Member. He (Martill, 1988, 1990a) considered the overgrowth or entanglement of carcasses by such a mat to enhance the fishes' preservational potential by 1) preventing the carcass from refloating; 2) providing a plentiful source of organic matter for autotrophic bacteria (which he considered fueled the complex diagenetic reactions); 3) by protecting the carcass from scavengers; and, 4) by enclosing the fish in its own decay-induced, low pH microenvironment thus stimulating the precipitation of apatite[1].

Actualistic mineralizing experiments performed by Briggs and Kear (1993a) strongly support the involvement of bacterial scums in the postmortem phosphatization of soft

[1]Note that contrary to Martill (1988), Allison (1988d) considered apatite to preferentially precipitate under at high pHs.

tissues. They, like Martill (1988) but contrary to Allison (1988d), suggested anoxia and a lowering of the pH in the vicinity of the carcasses (as a result of anaerobic decay) to be essential for mineralization. In Briggs and Kear's (1993a) experiments, these conditions were afforded by an air-tight aluminium bag; in the Romualdo Member, Martill (1988, 1990a) maintained prokaryotic mats played the same role.

Wilby and Martill (1992) have reiterated the importance of microenvironments to the postmortem phosphatization of soft tissues. In the Romualdo Member, exceptional anatomical details of decapod shrimps are preserved within the stomachs of certain fish, despite having been ingested. In contrast, those lying beyond the fish are only poorly preserved. Wilby (1993) also discussed the probable role of cellular and subcellular microenvironments in soft tissue phosphatization, and the importance of specific biomolecular templates within individual cells. Indeed, Wilby (1993) in support of Martill (1990a), proposed postmortem phosphatization of soft tissues to be related to certain processes of biomineralization. The importance of specific organic substrates as sites for the nucleation of apatite has also been recognised by Pinna (1985) and Schultze (1989).

Many workers have described phosphatized soft tissues which are entirely pseudomorphed by mineralized microbes (e.g. see Martin *et al.*, 1986; Willems and Wuttke, 1987; Micklich and Wuttke, 1988; Mehl, 1990; Allison, 1988d; Martill and Wilby, 1993a), and have thus invoked the direct involvement of the micro-organisms in their mineralization. Martin *et al.* (1986) proposed the soft tissues of Liassic ichthyosaurs to have been smothered by bacterial- or fungal-mats which subsequently became mineralized; whilst Willems and Wuttke (1987) and Micklich and Wuttke (1988) envisaged microbes replacing the soft tissues of Eocene fish and Permian amphibians respectively, to have created a microenvironment (reducing, pH 7-9) suited to the precipitation of phosphate ions liberated from the decomposing carcasses.

Hirschler *et al.* (1990a) have examined experimentally the role of microbes in the phosphatization of soft tissues. They emphasised the importance of microbially derived alkaline phosphatases in releasing organically-bound phosphates, and demonstrated that

these enzymes may induce the precipitation of apatite (from an organically-bound source of phosphorus and an inorganic source of Ca^{2+}) even in the absence of microbes. This led them (Hirschler *et al.*, 1990b) to conclude that although not preserved themselves, microbes may induce the precipitation of apatite by releasing phosphates from organics and by creating a suitable environment for the precipitation of apatite. In such "biologically induced" precipitation, the microbes do not participate *directly* in nucleating the crystallites. In contrast, in "biologically controlled" precipitation, the microbes both release the phosphate from the organic substrate, and provide the site for its nucleation. Hirschler *et al.* (1990b) considered this mechanism of mineralization to be a rarity.

An entirely different model for which little support has been given (although see Wuttke, 1991) has been proposed by Schultze (1989). On the basis of exceptionally well preserved phosphatized soft tissues in fish from an Upper Jurassic (Oxfordian) deposit in Northern Chile, he (Schultze, 1989) dismissed the 'traditional' syngenetic models of Müller (1985), Willems and Wuttke (1987), Martill (1988), and Allison (1988d) in favour of the soft tissues being impregnated with calcium phosphate during life. This, he proposed, had resulted from the breakdown of the fishes' calcium metabolism due to the ingestion of phytoplankton containing high levels of vitamin D_3 (a chemical known to provoke the unregulated mineralization of soft tissues in live cattle).

1.6.3 TIMING OF PHOSPHATIZATION

Dean (1902, 1909) believed the soft tissues of sharks from the Cleveland Shale to have been phosphatized soon after death. He (Dean, 1902, pp276-277) stated "the fresher the tissue to be fossilized, the better are its chances for accumulating phosphate", and that "during decomposition.....the petrifying tissues would have broken down *and would* hardly have been favourable for the deposition of accurate casts" (my italics).

Initially, the exceptional preservation and the apparent absence of any evidence for decay in the Orsten fauna of the Alum Shale Formation (Sweden) led Müller (1979) to suggest phosphatization had actually caused each organism's death. Later however, after recognising

certain taphonomic structures, he (Müller, 1985) proposed phosphatization to have occurred either at the time of death or immediately afterwards.

Similarly, Schultze (1989) was unable to identify any evidence of decomposition in the phosphatized soft tissues of fish from the Cordillera de Domeyko of Chile, and thus proposed mineralization had occurred in life.

In contrast, most other workers agree phosphatization to be a postmortem event. Willems and Wuttke (1987) considered the soft tissues of amphibians from Lake Odernheim (Lower Permian, Germany) to have taken place over a period of several weeks after death. This was necessary to permit a sufficient supply of Ca^{2+} to infiltrate the carcasses from the water column.

According to Martill (1990a) however, mineralization had been considerably more rapid than this in the Romualdo Member of NE Brazil. He (Martill, 1990a) suggested the exceptional state of preservation of soft tissues in the Romualdo Member would require either decomposition to have been arrested by some natural preservative (e.g. hypersaline water), or mineralization to have been extraordinarily rapid. Indeed, replication in actualistic taphonomic experiments of taphonomic structures preserved in the gills of fish from the Romualdo Member led Martill and Harper (1990) to propose mineralization to have taken place within 5hrs of death.

Recently however, Briggs and Kear (1993a) have questioned the validity of the experimental conditions used by Martill and Harper (1990). Briggs and Kear (1993a) based on the production of phosphatized soft tissues *in vitro*, proposed mineralization to be initiated 2 weeks after death and to continue for at least 6 weeks thereafter. Much like Reis (see Dean, 1902, pp 276-277), they (Briggs and Kear, 1993a) envisaged phosphatization of soft tissues to depend on a critical balance between decay (required to release phosphate ions from the carcass and to establish a favourable microenvironment) and precipitation (necessary to preserve the soft tissues before too much information is lost).

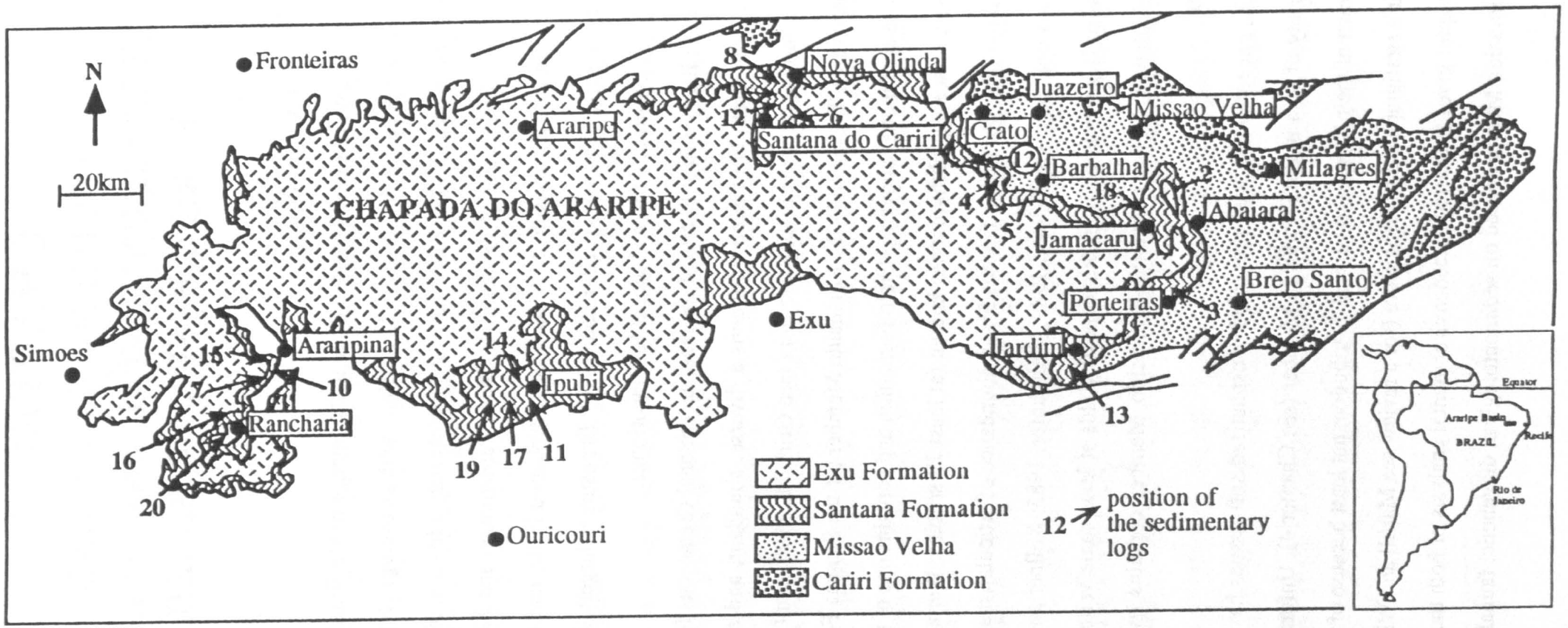
CHAPTER 2
SEDIMENTOLOGY AND PALAEOENVIRONMENTAL ANALYSIS OF THE ARARIPE BASIN, NE BRAZIL

2.1 INTRODUCTION

The Araripe Basin is a fault-bounded basin in the interior of NE Brazil dominated by an east-west trending plateau - the Chapada do Araripe - with an average altitude of 800m. The Chapada covers an area of ≈17500km^2 (almost certainly only a fraction of its former extent) and straddles the boundaries of the states of Ceará, Pernambuco and Piaui (text fig. 2.1). The oldest sediments are considered to have been deposited in the latest Jurassic, and the youngest in the early Cenomanian (Berthou, 1990). Exposure is largely limited to seasonal stream cuttings, gypsum mines, and illegal fossil workings.

The Araripe Basin is host to two of the most exceptional Cretaceous fossil assemblages. The oldest - the Crato Formation - contains a diverse, abundant, and exceptionally well preserved Gondwanan entomofauna and flora (see Grimaldi, 1990, and Crane and Maisey, 1991 respectively). The second - the Romualdo Member (of the Santana Formation) - occurs only a few tens of metres above the Crato Formation and contains one of the most abundant and diverse Cretaceous ichthyofaunas known (at least 19 species, Wenz and Brito, 1990). The Romualdo Member is also famous for an abundance of articulated and well preserved pterosaur material (e.g. see Kellner, 1989; Wellnhofer, 1985; Kellner and Campos, 1990). Almost every large concretion in this deposit yields at least some vertebrate material. A simple calculation suggests that the Romualdo Member contains over 1.225×10^{11} concretions!

Recently, exceptionally well preserved phosphatized soft tissues have been recovered from a variety of taxa in the Romualdo Member (see Chapter 4). Unfortunately however, little is known of their lateral and vertical distribution, not least because of the confused state of the basin's stratigraphical nomenclature (for a review see Martill and Wilby, 1993b, Table 1). This chapter gives a detailed palaeoenvironmental analysis of both the Crato Formation and the Romualdo Member based on sedimentological, diagenetic, faunal, and taphonomic

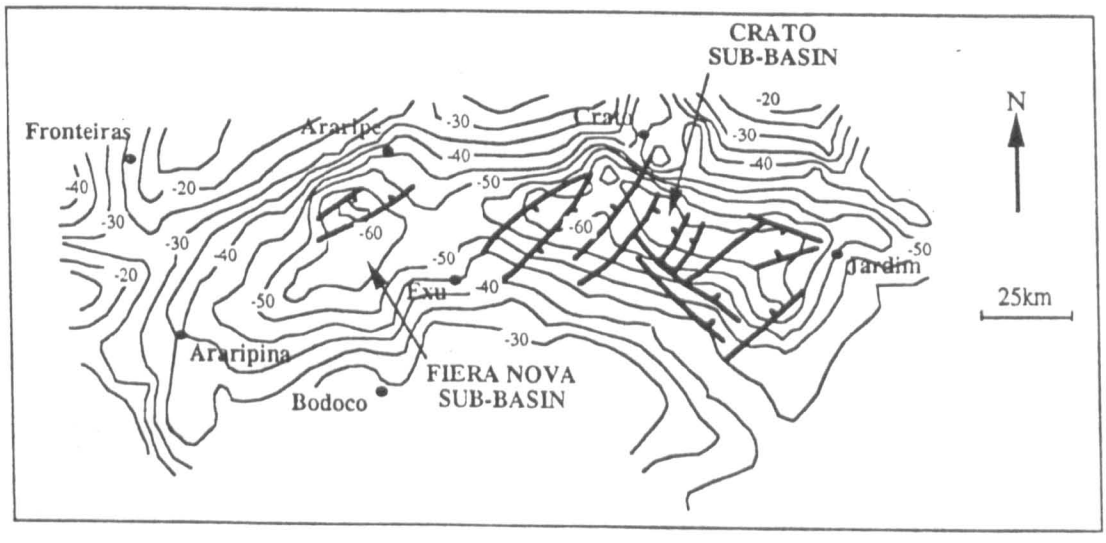


Geological map of the Chapada do Araripe. The position of major towns and all of the stratigraphical logs measured are given.

criteria. In particular, the palaeosalinity, oxygen level, temperature, and sediment consistency of the Romualdo Member are examined. I also discuss the distribution of phosphatized soft tissues in the Araripe Basin.

2.2 STRUCTURAL EVOLUTION OF THE ARARIPE BASIN

The sediments of the Araripe Basin unconformably overlie Proterozoic metasediments and intrusives of the Piancó-Alto Brigida orogenic belt in the Borborema structural province (see Brito Neves, 1990). They were deposited in a series of NE-SW trending horsts and grabens (text fig. 2.2) bounded to the north by the Paraiba lineament, and to the south by the Pernambuco lineament (text fig. 2.3). Erosional remnants to the south indicate the basin to have had a greater extent than its present outcrop, and small sub-basins to the north may also have been linked to the Araripe Basin (Berthou, 1990). The presence of a topographic high to the west however, probably prevented the basin from extending much further in that direction than is suggested by its present outcrop (Moraes *et al.*, 1976).

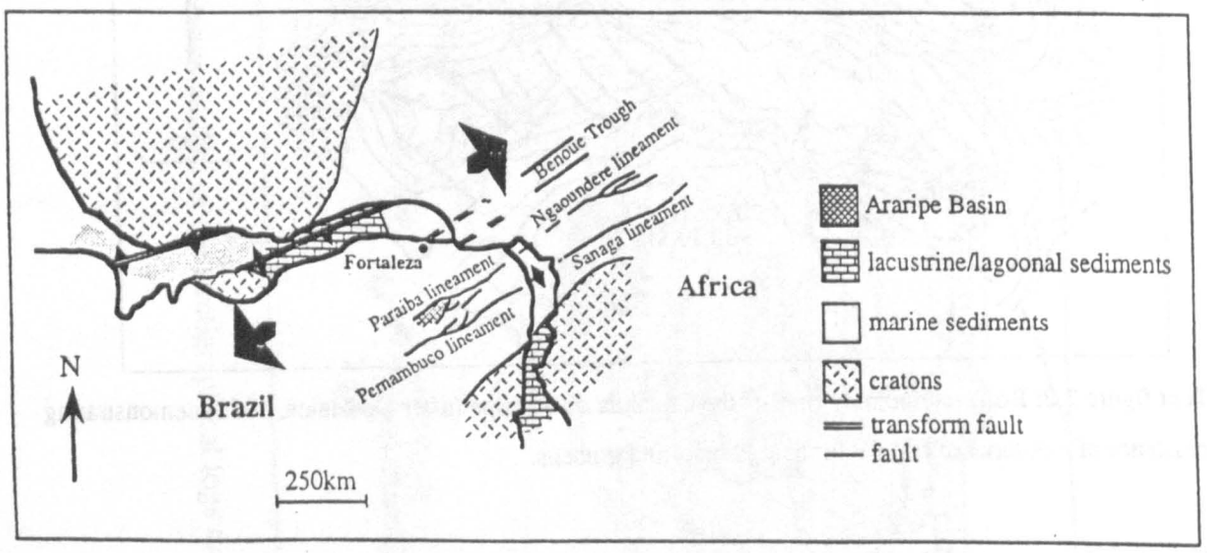


Text figure 2.2: Bouguer anomaly map of the Chapada do Araripe (after De Matos, 1988) demonstrating the existence of a number of NE-SW trending horsts and grabens.

The development of the Araripe Basin was initiated by the break-up of the Gondwanan super-continent in the late Jurassic, and growth of the South Atlantic Ocean during the Cretaceous (text fig. 2.4). Separation of NE Brazil from Africa began in the early Cretaceous; the oldest oceanic crust in this region being Albian in age (Berthou, 1990). The shape and structural behaviour of the Araripe Basin, and the transform faults of the South Atlantic Ocean, are a legacy of ancient basement heterogeneities (Brito Neves, 1990). The

Pernambuco and Parnaiba lineaments defining the Araripe Basin may be traced across the Atlantic, and are equivalent to the Sanaga and Ngaoundere faults of the West African Shield (Benkhelil, 1988; Berthou, 1990).

Text figure 2.3: Cretaceous sedimentary basins of NE Brazil. The Araripe Basin is bounded to the north and south by major lineaments (based on Berthou, 1990).



Text figure 2.4: The relative positions of NE South America and West Africa during the Aptian. The shape and structural behaviour of the Araripe Basin is dictated by ancient basement heterogeneities (modified after Berthou, 1990).

Tensional stresses imposed by dextral strike-slip movements on the Paraiba and Pernambuco lineaments were probably responsible for the progressive westward migration of the basin's depocentre (Silva, 1983, p234, Berthou, 1990, p113).

Continental break-up initiated widespread rifting across much of the rest of NE Brazil, and created a number of other basins coeval with the Araripe Basin (text fig. 2.3; see Petri, 1987 for a review). The tectonic and sedimentary histories of some of these are very similar (Brito and Campos, 1982, 1983). Short-lived marine episodes in the Araripe Basin (Hashimoto *et al.*, 1987; Baudin *et al.*, 1990; Berthou *et al.*, 1990; Berthou and Pierre, 1990; Pons *et al.*, 1990), and shared faunal elements with other basins (see Silva Santos, 1991 for a review), indicate communications to have existed between these, the Araripe Basin, and the proto-Atlantic Ocean. It is however, a matter of considerable debate as to whether these connections existed with the central Atlantic via the Potiguar or Parnaiba Basins (Braun, 1966; Hashimoto *et al.*, 1987; Berthou, 1990; Mediros, 1990; Regali, 1990), or the South Atlantic via the Reconcavo-Tucano-Jatoba aulacogen complex or the Sergipe-Alagoas Basin (Mabesoone and Tinoco, 1973)(text fig. 2.3).

2.3 AGE OF THE SEDIMENTS IN THE ARARIPE BASIN

Except for some early (?Silurian) pre-rift sands and grits at the base of the sequence (the Cariri Formation, see Section 2.4.1), the Araripe Basin is generally considered to have developed in the middle to late Jurassic, and to have continued receiving sediment until the late Cretaceous (Berthou, 1990, fig. 3). However, due to the rarity of diagnostic fossils, and the absence of interbedded lavas or ashes (Martill, 1990b), the age of most of the sediments of the Araripe Basin (and other coeval basins) remains poorly constrained. This prompted Brito and Campos (1982, 1983) to erect a number of local stages for NE Brazil based on shared tectonic and sedimentological characteristics. Recently however, the validity of local stages has been questioned since there is little evidence to suggest that the development of the inland basins (e.g. the Araripe Basin) was precisely coeval with those of the continental margin (see Maisey, 1991 for a discussion). The validity of a "*Vinctifer* Biozone" which had been proposed by Brito (1984) is also in some doubt (Martill, 1988; Maisey, 1991); the

distribution of this fish is patchy, its range (particularly its lower limit) is poorly determined, and it is difficult to distinguish it from other aspidorhynchids.

Clearly, much work remains to be done regarding the precise age and correlation of these sediments.

2.4 STRATIGRAPHY AND PALAEOENVIRONMENTAL ANALYSIS OF THE ARARIPE BASIN (see Martill, 1993a for a palaeoenvironmental analysis of the sequence and Martill and Wilby, 1993b for a detailed discussion of its sedimentology).

The sediments of the Araripe Basin were first documented by Small (1913) who described a diverse assemblage of clastics, evaporites and carbonates between two units of coarse red sandstone. There has been little agreement over the nomenclature or position of boundaries within this sequence, and the basin's stratigraphy is thus in a state of considerable confusion (Beurlen, 1962, 1963, 1971; Santos and Valenca, 1968; Lima, 1979a; Silva, 1983, 1986a, 1986b; Brito, 1990; Ponte and Appi, 1990; Martill and Wilby, 1993b). At present no single scheme adequately reflects the complexity of the basin's sedimentary history, and until recently (Martill and Wilby, 1993b), type sections had not been formerly designated, nor the precise position of many boundaries defined.

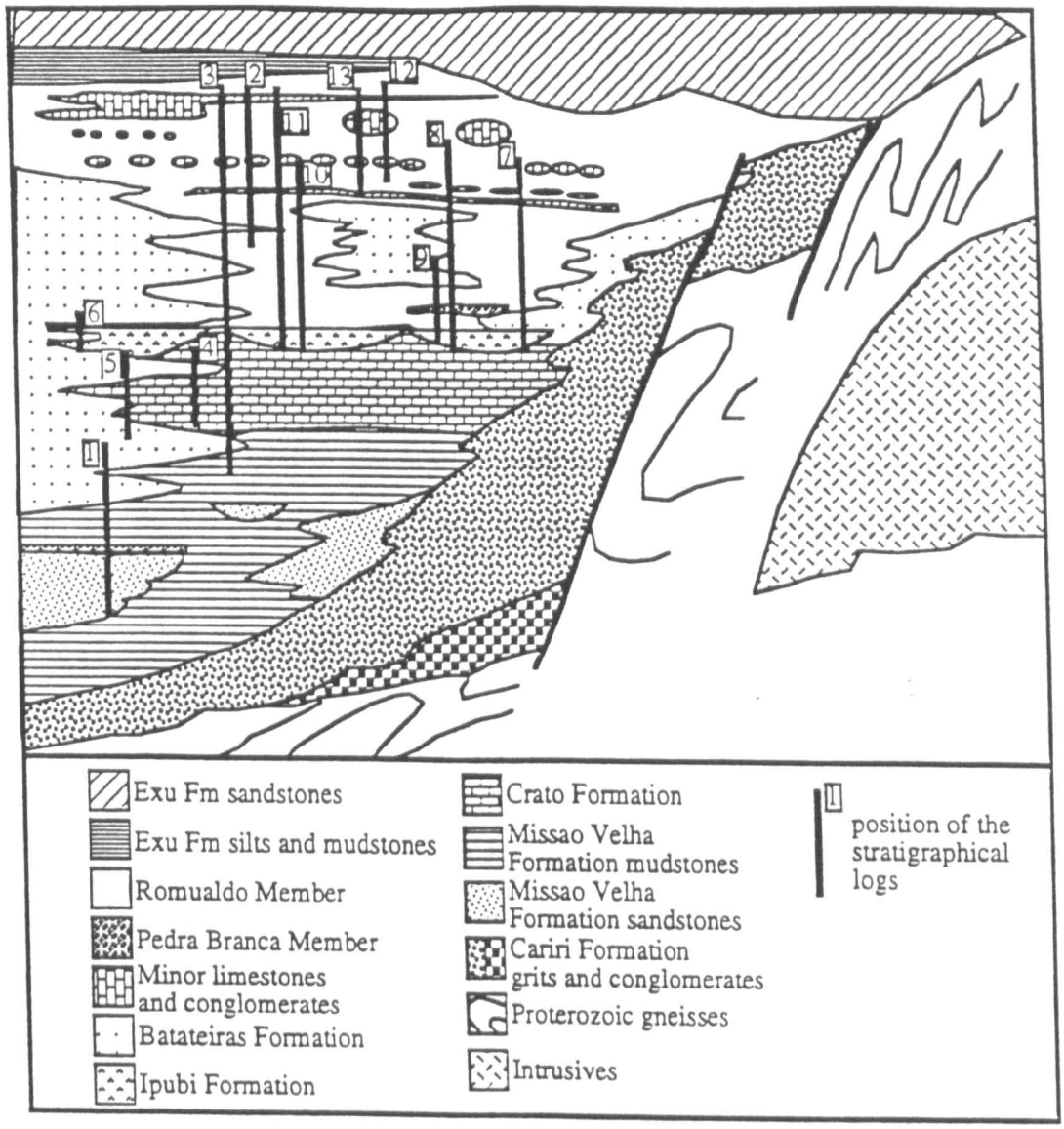
I present below a review of the stratigraphy of the Araripe Basin based on Martill and Wilby's (1993b) nomenclature. Stratigraphical logs not referred to directly in the text are given in Appendix 2. The stratigraphical position of logs referred to in the text is given in text figure 2.5, and their geographical locality is given in text figure 2.1.

Martill and Wilby (1993b) have divided the sequence into seven formations. These are:

2.4.1 THE CARIRI FORMATION: SILURIAN (Medeiros, 1990).

The Cariri Formation consists of 10-86m (Beurlen, 1963; Veiga, 1966; Ponte and Appi, 1990) of light coloured, cross-bedded, coarse sandstones, angular grits and conglomerates which lie unconformably on Proterozoic basement (see text fig. 2.5). The sediments are devoid of fossils and represent fluvial channel fills and fanglomerates derived from local source areas to the north and north-east (Medeiros, 1990). These sediments are well exposed

in the vicinity of Missao Velha (see text fig. 2.1) and occur beneath younger sediments in the north and south of the Chapada (Arai and Coimbra, 1990). At Juazeiro do Norte, grits referable to the Cariri Formation lie to the north of the Paraiba Lineament and appear to be truncated by this structure. The Cariri Formation is therefore almost certainly pre-rift.



Text figure 2.5: Interrelationships of the various lithological units of the Araripe Basin, and the relative position of the stratigraphical logs (after Martill and Wilby, 1993b).

2.4.2 THE MISSAO VELHA FORMATION: HAUTERIVIAN - UPPER APTIAN (Berthou, 1990).

The Missao Velha Formation consists of up to 740m (Ponte and Appi, 1990) of red, lenticular masses of coarse sandstones, grits and conglomerates interbedded with micaceous, red and occasionally green silts and clays (locality 1, text fig. 2.6). At Barbalha (see text fig. 2.1), algal limestones occur, and in an area of 'bad-lands' between Abaiara and Missao

Velha, silicified logs are abundant. Braun (1966) recorded the presence of ostracodes (see also Depeche *et al.*, 1990) and at least three species of conchostracans. The teeth of *Hybodus* sp., the teeth and scales of *Lepidotes* sp., skull bones of a large coelacanth, and a variety of indeterminate reptile teeth have also been collected from channel lags to the east of Missao Velha. Fossils in general however, are rare (pers. comm. Brito, 1991, Natural History Museum, Paris).

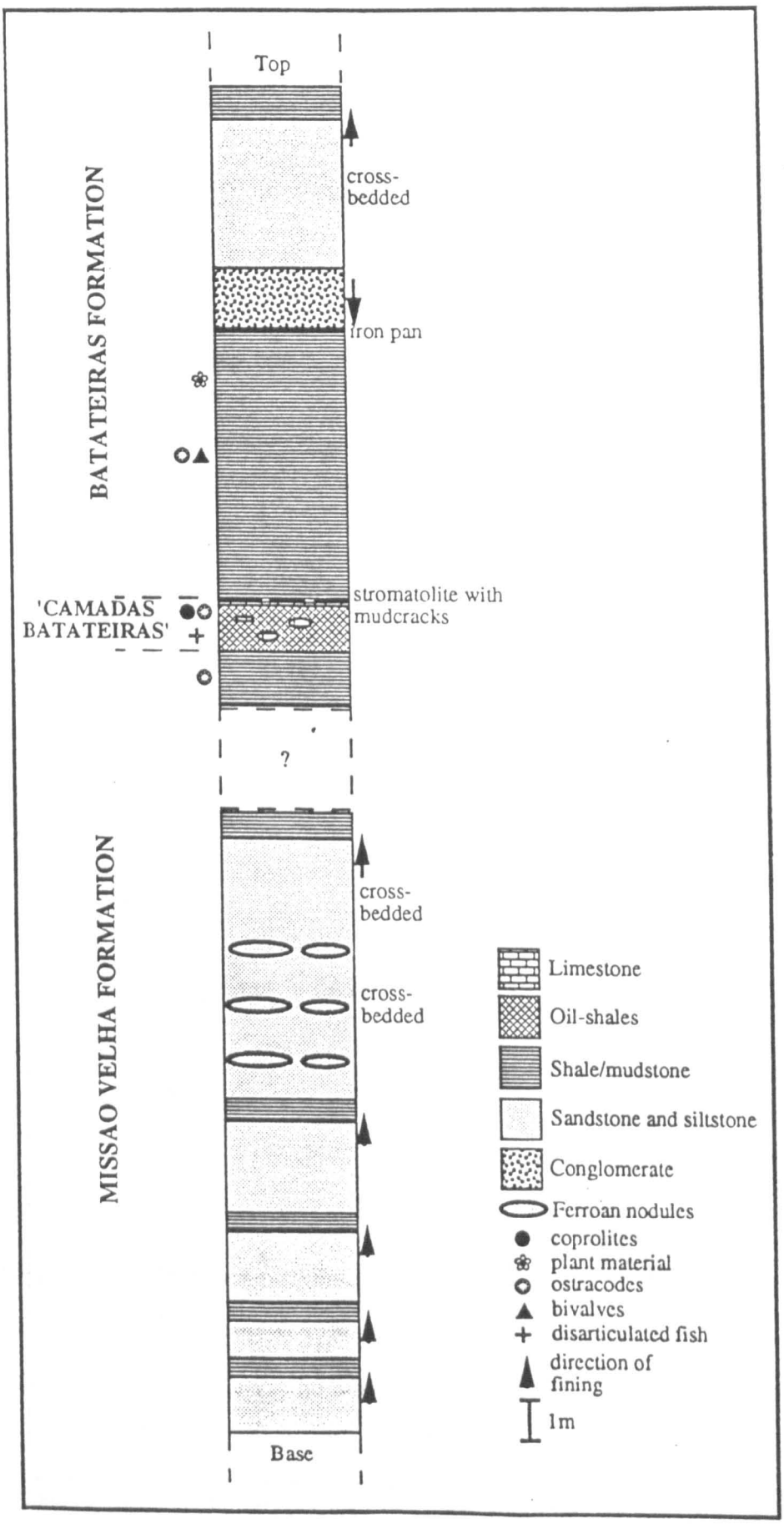
At the very top of the Missao Velha Formation, a marine (Hashimoto *et al.*, 1987; Baudin *et al.*, 1990; Pons *et al.*, 1990) bituminous paper shale (≈1m thick) termed the "camadas batateiras" (Berthou, 1990) occurs. This contains abundant phosphatic faecal pellets, ostracodes, and laterally impersistent, compressed carbonate concretions (see Berthou *et al.*, 1990, figs. 2, 4, 6 for typical appearance in thin section). The camadas batateiras was deposited within a confined, calm, and anoxic lagoon, probably in the still waters between drowned deltas (Berthou, 1990). It represents the first marine incursion into the Araripe Basin (Hashimoto *et al.*, 1987).

The Missao Velha Formation represents a complex interdigitation of coeval point bars and channel fills, with flood plain and lacustrine deposits (see text fig. 2.5).

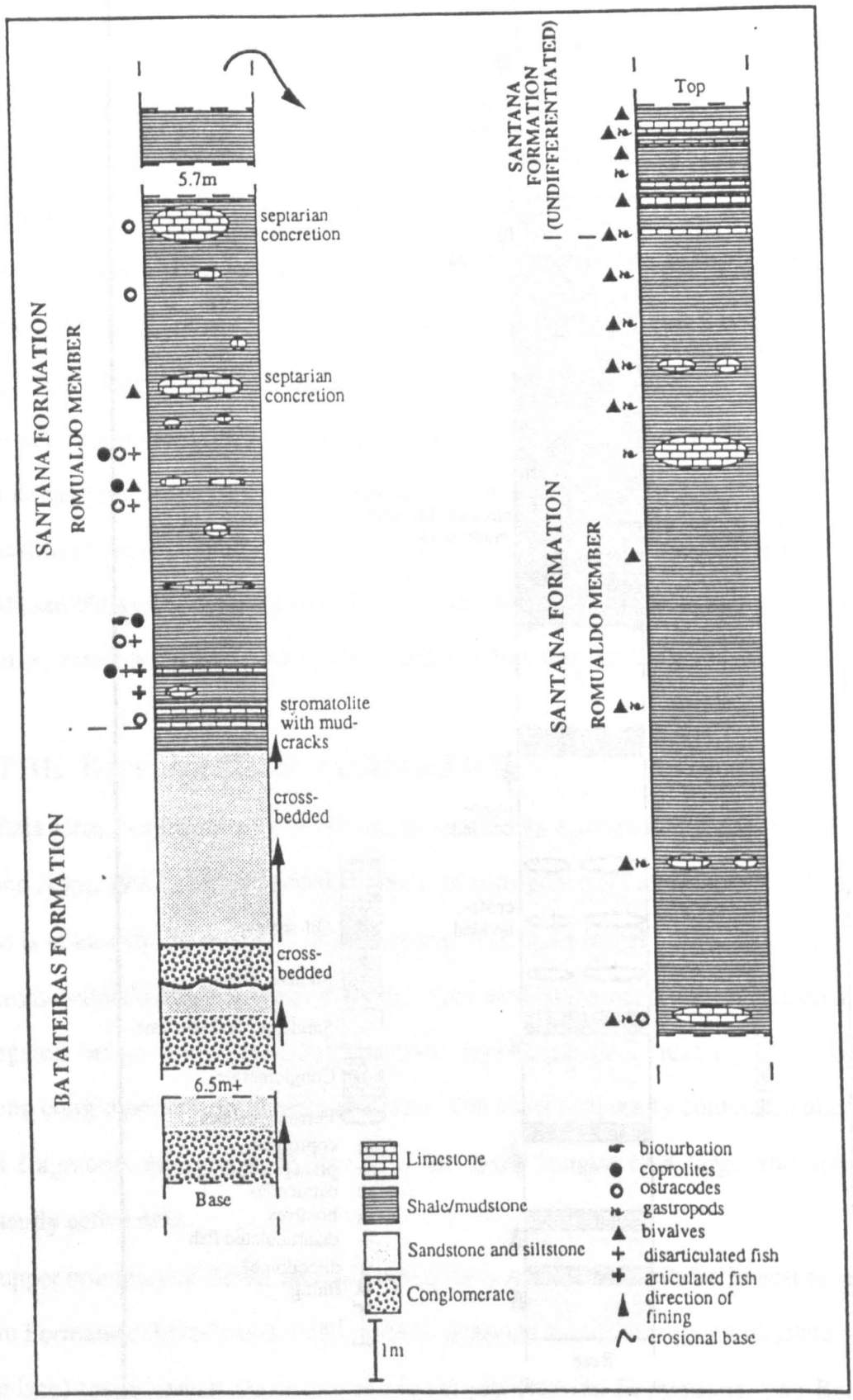
2.4.3 THE BATATEIRAS FORMATION

The Batateiras Formation marks the beginning of a new cycle of fluvial sedimentation (Ponte and Appi, 1990), rejuvenated by a phase of subsidence (Medeiros, 1990). Its precise thickness is unknown and probably varies greatly. It consists largely of packages of upward fining, cross-bedded sandstones and siltstones, each several metres thick, with intercalations of variegated brown and grey siltstones and clays (locality 2, text fig. 2.7). Upward coarsening conglomerates are locally abundant. The clays frequently contain an abundance of plant fragments and probably represent the distal tongue of a large and somewhat intermittently active delta.

The upper boundary of the Batateiras Formation is gradational with the lowest member of the Crato Formation (Martill and Wilby, 1993b) although locally it may interdigitate with (or even replace) these limestones and/or the Ipubi and Santana Formations. The Batateiras Formation reaches its greatest thickness in the north and far east of the Araripe Basin.



Text figure 2.6: Sedimentary log of locality 1, Rio Batateiras, Cascata near Crato.



Text figure 2.7: Sedimentological log of locality 2, Sierra do Moazina, near Abaira.

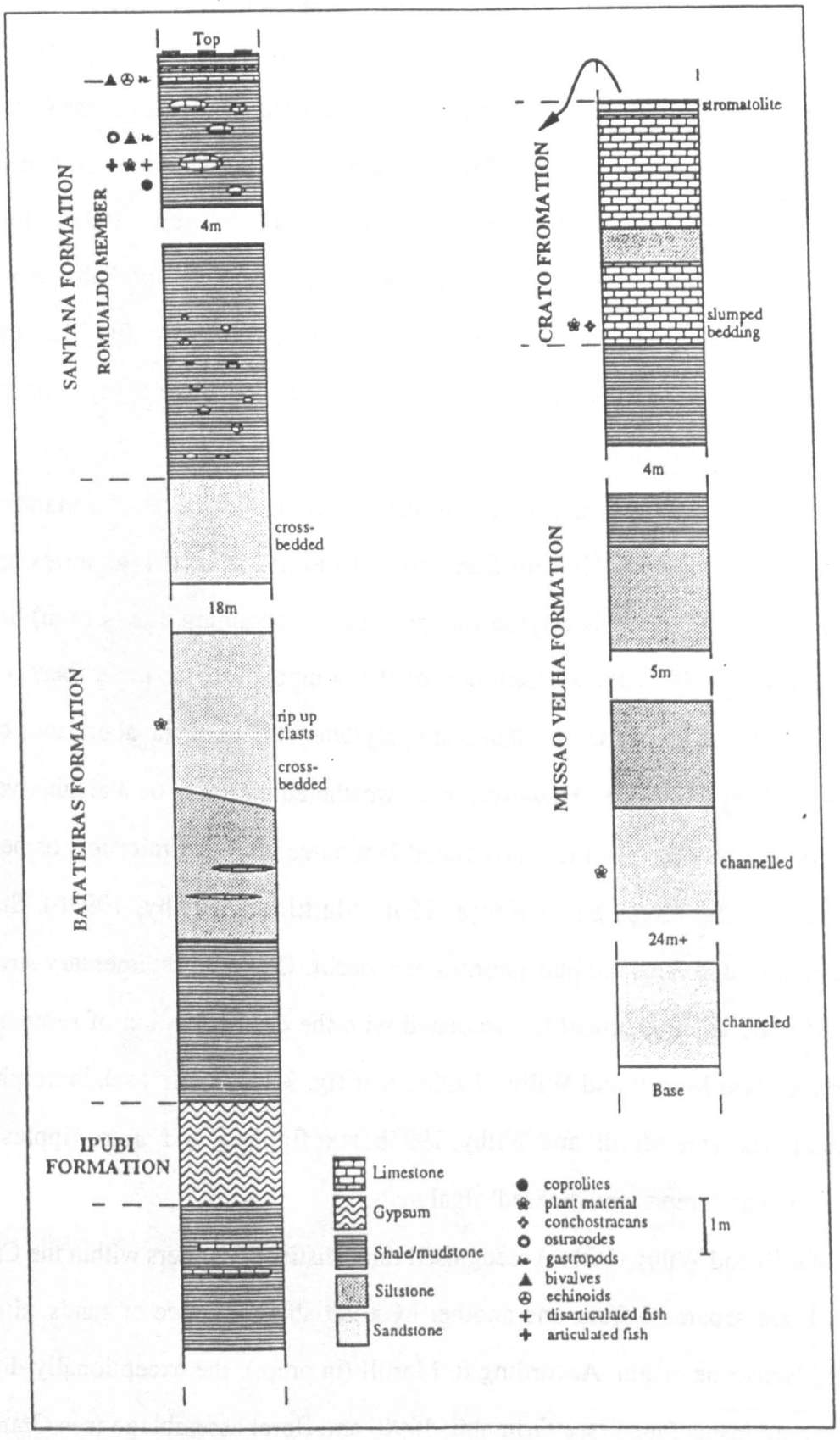
2.4.4 THE CRATO FORMATION: UPPER APTIAN - LOWER ALBIAN

(Pons *et al.*, 1990).

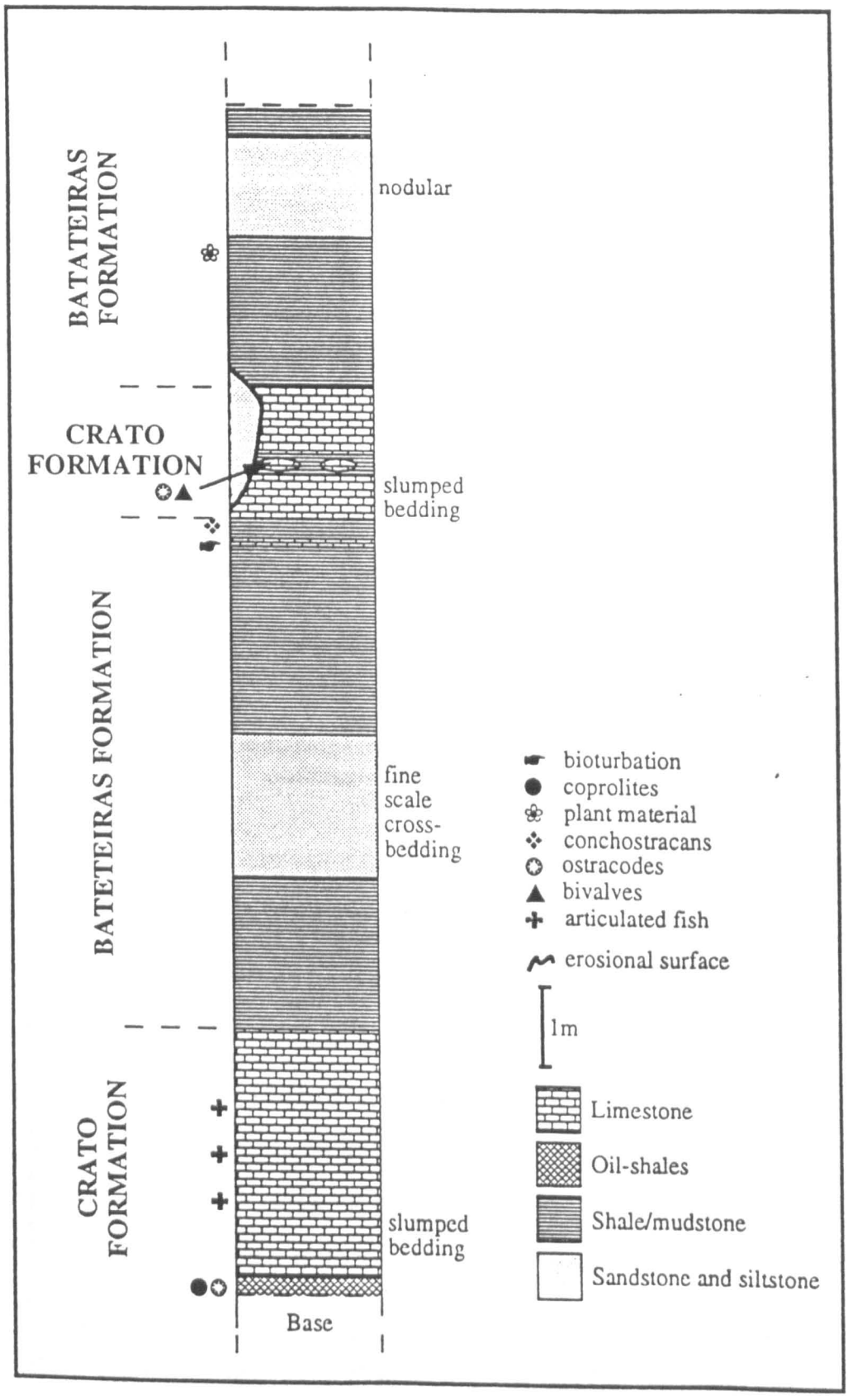
The Crato Formation consists of 30m to 80m (Berthou, 1990; Ponte and Appi, 1990) carbonate-rich laminates. Near Crato at Cascata (locality 1), at Porteiras (locality 3, see text fig. 2.8), Jardim (pers. comm. Martill), and at Jamacaru, there is a gradual transition from the shales of the Batateiras Formation to the laminated carbonates of the Crato Formation. In contrast, at Tatajuba (3km east of Nova Olinda) and SW of Jardim, the plattenkalks lie with marked unconformity directly on basement (Martill, in prep.) and are interbedded with conglomerates containing clasts of quartzite and gneiss. These probably represent shoreline facies and fanglomerates derived from local topographic highs. The top of the Crato Formation is marked at Porteiras (locality 3, see text fig. 2.8) by a partially silicified, brecciated stromatolite.

There is some vertical variation in the character of the Crato Formation but very little difference between equivalent horizons at localities several kilometres apart. The most characteristic lithology is a pyrite-rich plattenkalk containing thin (<1mm) organic-rich (see Baudin *et al.*, 1990 for a discussion of the composition) laminae (pers. comm. Martill, 1992). When fresh, these limestones are grey/blue, reflecting the abundance of disseminated FeS_2 and organic matter. However, when weathered they take on a distinctive cream/orange colour. Elsewhere, pisolites, brecciated laminates, massive micrites, or pelites dominate (Braun, 1966; Santos and Valença, 1968; Martill and Wilby, 1993b). Silicified domal stromatolites and peletal phosphorites also occur. Common sedimentary structures include small scale slumps, probably associated with the destabilization of sediments on uneven surfaces (see Martill and Wilby, 1993b, text fig. 3.11), halite pseudomorphs with hopper crystal faces (see Martill and Wilby, 1993b, text fig. 3.9), and micro-ripples (fig. 2.1). The latter probably represent 'creased' algal mats.

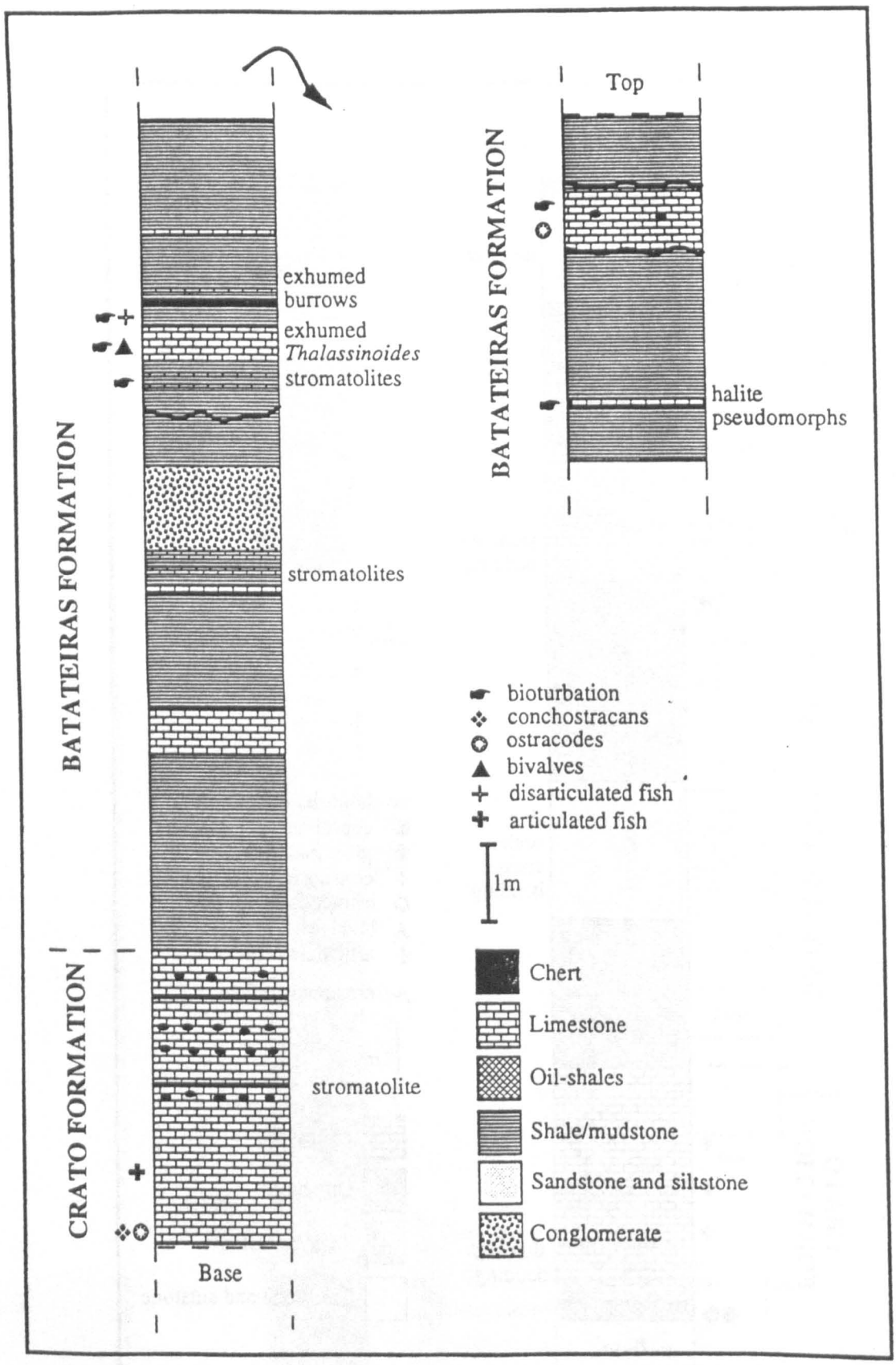
Martill and Wilby (1993b) recognised three distinct members within the Crato Formation which are separated from one another by a variable sequence of sands, silts and clays of delto-lacustrine origin. According to Martill (in prep.), the exceptionally diverse and well preserved insect fauna (see Grimaldi, 1990) and floral assemblage (see Crane and Maisey, 1991) is most prolifically represented in the lowest member - the Nova Olinda Member.



Text figure 2.8: Sedimentological log of locality 3, Porteiras.



Text figure 2.9: Sedimentological log of locality 4, Mina IBACIP, NW of Barbalha.



Text figure 2.10: Sedimentological log of locality 5, Old Mina IBACIP, south of Barbalha.

The limestones of the Crato Formation are locally replaced (particularly in the east) by deltaic sands, silts and mudstones referable to the Batateiras Formation (for the stratigraphical relationship of these units in the NE of the basin, see Martill and Wilby, 1993b, text fig. 3.5). At Porteiras (locality 3, see text fig. 2.8), the Crato Formation is divided into two by 30cm of silty shales containing plant material and conchostracans. At Mina IBACIP (locality 4, text fig. 2.9), lobes of finely cross-bedded sandstone and micaceous shales containing conchostracans and plant material interdigitate with, and laterally replace the plattenkalks. Fifteen kilometres further south (locality 5, text fig. 2.10), an unknown thickness of the Crato Formation (and all of the Ipubi Formation, see below) is replaced by grey fissile shales and a 1.2m thick, matrix-supported conglomerate containing angular quartz-, micrite-, and mudstone-clasts. These sediments represent the repeated invasion of the 'Crato Lagoon' by deltaic lobes assignable to the Batateiras Formation.

The importance of the Batateiras deltaic sediments probably diminishes with distance from the basin's margins; a more complete sequence of carbonates and shales almost certainly occurs towards the centre of the basin.

2.4.4.1 FAUNA AND FLORA OF THE CRATO FORMATION (see Grimaldi, 1990, Maisey, 1991, and Martill, 1993a for a review)

The Nova Olinda Member of the Crato Formation contains probably the most important mid-Cretaceous insect fauna presently known. Insects are extremely abundant, well preserved (fig. 2.2) and very diverse (around 20 Orders); some being the oldest recorded representatives (e.g. the ant described by Brandão *et al.*, 1989). Examples of Orthoptera, Ephemeroptera, Coleoptera, and Blattodea are particularly abundant. In addition, spiders, scorpians and decapod crustaceans have been recorded (see Maisey, 1991), and ostracodes and conchostracans are abundant.

The Crato Formation also contains an abundant macroflora. This has recently been reviewed by Crane and Maisey (1991) and the microflora has been described by Lima (1978a, b; 1979a; 1980) and Pons *et al.* (1990). According to Martill (in prep.), this too is restricted largely to the Nova Olinda Member. It is dominated by *Brachyphyllum* sp. (a succulent gymnosperm)(see fig. 2.16) and reed-like leaves, although various seeds, fruits,

stems (fig. 2.3) and leafs of other plants are not uncommon. Fine examples of these are figured by Crane and Maisey (1991). In addition, at a number of horizons in the Nova Olinda Member, enormous numbers of a simple ?algal filament (≈2mm x 7mm) occur (fig. 2.3).

In contrast, with the exception of juveniles of the gonorhynchiform fish - *Dastilbe elongatus* (fig. 2.4) - which occur in large numbers as mass mortalities at certain horizons in the Nova Olinda Member, the vertebrate fauna is relatively impoverished. Adults of *D. elongatus*, and specimens of *Cladocyclus* sp. and *Tharrias* sp. do occur, but are extremely rare. A feather (Neto and Kelner, 1988) and a frog (Kellner and Campos, 1985) have also been recorded. Notable absentees are pterosaurs which are relatively abundant higher up the section, and which are known from similar lithologies elsewhere (e.g. the Solnhofen Limestone, see Barthel *et al.*, 1990). Similarly, fully terrestrial vertebrates such as snakes and lizards are extremely rare. Martill (in prep.) proposed that since the quality of preservation of organisms in the Crato Formation is so exceptional (see below), the absence of certain groups of organisms is most likely an ecological phenomenon.

The entomofauna of the Crato Formation is particularly well preserved; the majority of specimens are fully articulated, and eye facets, gills, and setae are preserved in exceptional three-dimensional detail (see Grimaldi and Maisey, 1990, fig. 5). Grimaldi and Maisey (1990) considered these to be replaced by goethite. However, examination of material from unweathered sections indicates goethite to be secondary after pyrite. Martill (pers comm.) has noted that fresh fossil material is also extremely rich in organics. In addition, I have identified phosphatized soft tissues in one specimen of *D. elongatus* (PRW/17) and Martill (pers. comm., 1992) has identified them in one insect.

2.4.4.2 PALAEOENVIRONMENTAL INTERPRETATION OF THE CRATO FORMATION

Most palaeoenvironmental analyses of the Crato Formation have concluded the limestones to have been deposited in shallow, landlocked, saline lakes (e.g. Mabesoone and Tinoco, 1973; Silva, 1983; 1986a, 1986b; Grimaldi and Maisey, 1990, p13), although

Moraes *et al.* (1976) proposed the repeated intercalation of carbonate and siliciclastic facies to be more characteristic of a tidal lagoon. Silva (1983; 1986a, b) proposed the Crato Lagoon to have been hypersaline and to have become progressively more saline until evaporitic facies finally developed (i.e. the Ipubi Formation, see below). In contrast, Grimaldi and Maisey (1990) envisaged a rather more hospitable environment to have existed in which fish fry and adult and nymphal aquatic insects (e.g. Ephemeroptera, Odonata, Heteroptera) lived in abundance. This scenario however, is supported by very little evidence. Indeed, postmortem contortion of the vertebral column of over 50% of all specimens of *Dastilbe* (see fig. 2.4) in a manner consistent with dehydration, suggests (along with the lack of bioturbation) the bottom waters of the Crato Lagoon to have been hypersaline.

Martill and Wilby (1993b, text fig. 3.10) similarly interpret the Crato Lagoon to have been extremely hostile. Martill (in prep.) demonstrated that fully aquatic insects (except for the larvae of Ephemeroptera) are actually very rare, and considered all of those present to have been washed in from local river systems. Under more amenable conditions, a much more diverse aquatic biota would have existed (Martill, in prep.). From a random sample, he (Martill, op. cit.) demonstrated the entomofauna to be dominated by strong flying and 'hopping' insects such as the Orthoptera, Odonata and Ephemeroptera which presumably landed accidentally on the water surface. Incorporation into the water column during life is supported to some extent by the abundance of specimens with open wing cases (pers. comm. Martinez-Delclos, Barcelona, 1991).

The rarity of adult fish, and the abundance of juvenile specimens of *Dastilbe* in mass mortalities is also consistent with the Crato Lagoon having been a rather hostile environment. The juvenile fish were probably washed from local river systems into the hypersaline lagoon where they subsequently perished from salinity stress. Martill (in prep.) proposed adults of the same species, being rather stronger swimmers, were able to avoid being washed in, and hence are not as well represented.

The presence of conchostracans (Mabesoone and Tinoco, 1973), freshwater ostracodes (Silva-Telles and Viana, 1990), and *Thalassinoides* at certain levels within the Crato Formation, indicates the inhospitable conditions to have been occasionally interrupted.

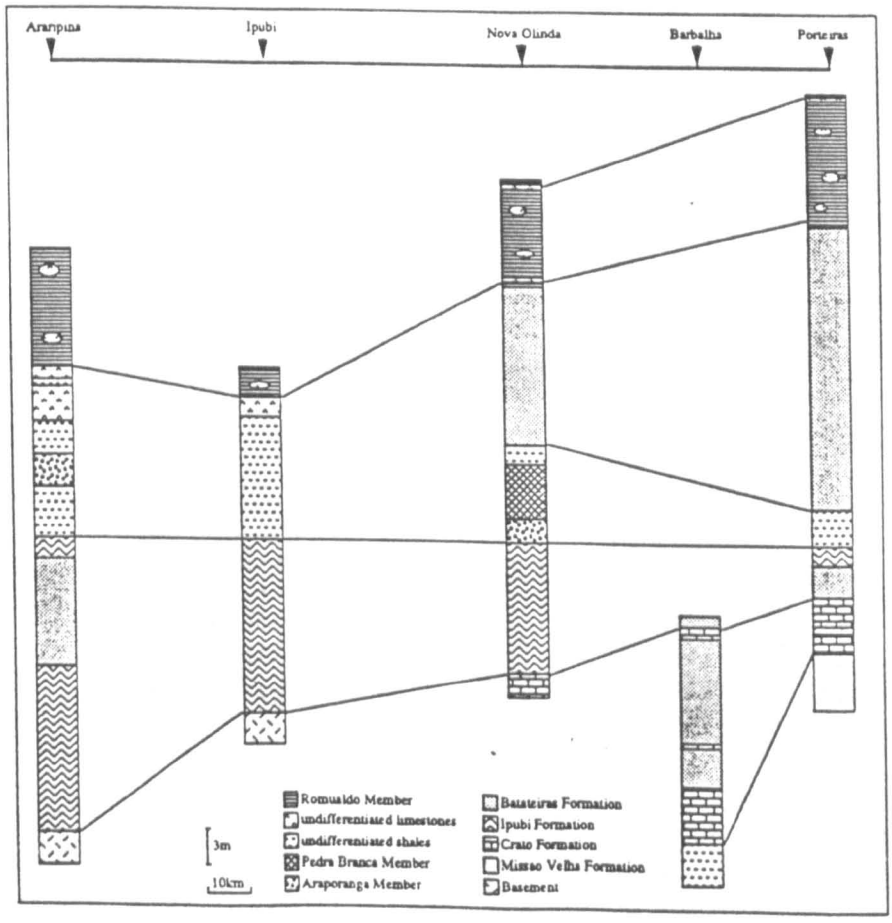
According to da Silva-Telles and Viana (1990), these horizons correspond to short-lived oxygenation events. Certainly, the pyritization of plant and insect material, and the abundance of disseminated pyrite throughout most of the sequence, indicates anoxic porewaters to have prevailed in the Crato lagoon. However, the close association of conchostracans, ostracodes, and trace fossils with deltaic sediments of the Batateiras Formation, suggests population of the substrate to have been predominantly salinity controlled. I propose that large influxes of freshwater from the Batateiras delta system permitted the localized development of 'pools' in which salinities were reduced to levels tolerated by certain organisms.

Martill (in prep.) in contrast to most workers (e.g. Silva, 1983; Berthou *et al.*, 1990; Grimaldi and Maisey, 1990) considers the Crato Formation may have had a marine influence (albeit limited) which increased up section as the connection to the open sea developed. Although I support this model, evidence for a marine influence is largely circumstantial. Firstly, there is a clear progression in the degree of marine influence up section in the sediments of the Araripe Basin from the fluvial/lacustrine beds of the Missao Velha Formation, through the dominantly saline waters of the Crato and Ipubi Formations (see below), to the Santana Formation in which definite marine signatures have been identified (see below). Since at least one marine incursion occurred in the Missao Velha Formation (see above), and Berthou and Pierre (1990) have established the Ipubi Formation to have been deposited under a marine influence, it is not unreasonable to assume marine water to have periodically invaded the Crato lagoon. Indeed, the abundance of disseminated pyrite in the plattenkalks of the Crato Formation, and thus the presence of sulphate (essential for pyrite production), implies a marine connection. Typical marine faunas may have been prevented from becoming established by the limited nature of the connection, and by the periodic influxes of large volumes of freshwater. These two factors together with the lagoon's restricted nature would have made it susceptible to dramatic fluctuations in salinity.

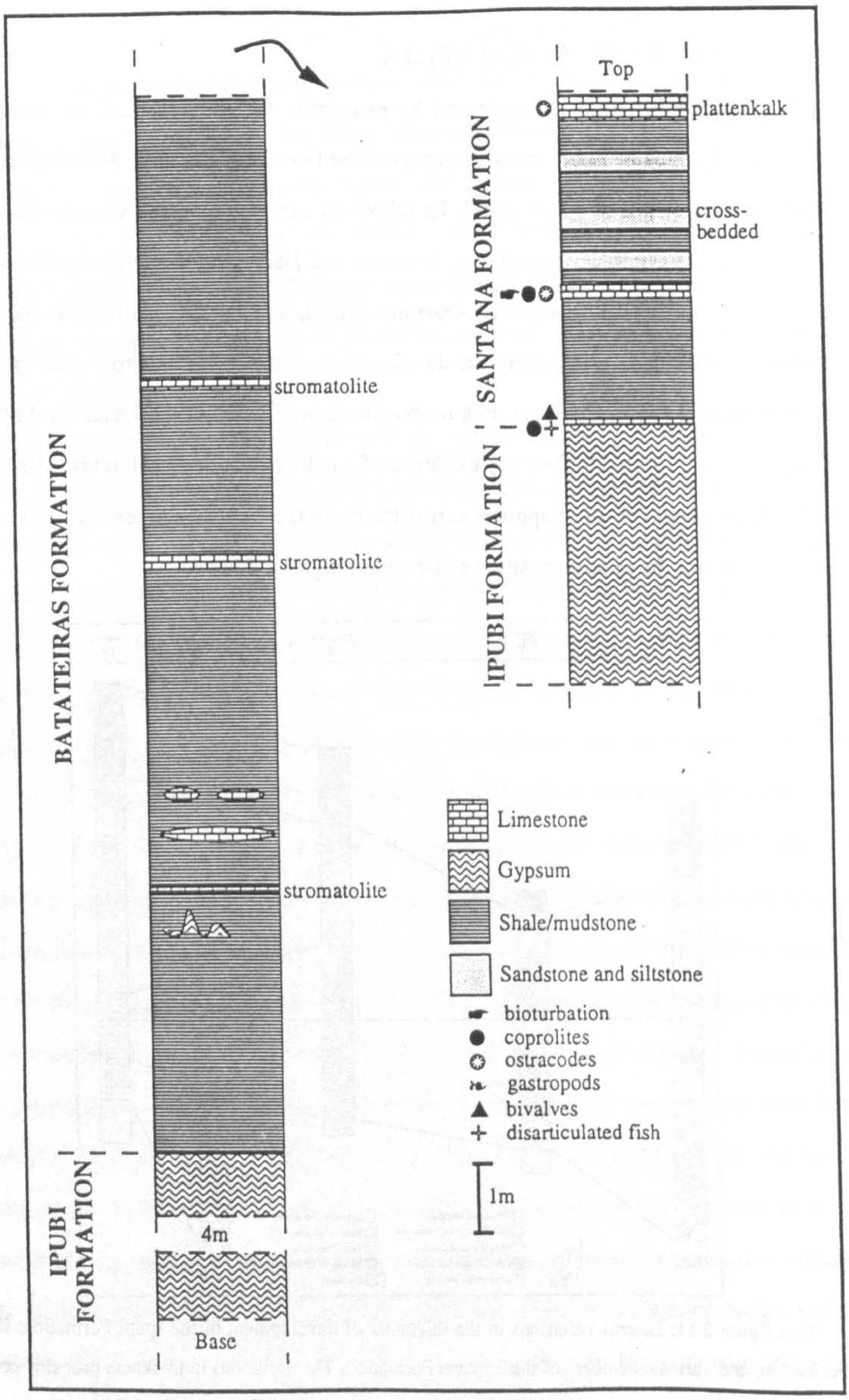
The second piece of circumstantial evidence for a marine influence is the sudden widespread development of carbonates in an up until then, terrestrially dominated sequence. I propose their deposition to have been provoked by a marine incursion which was of sufficient magnitude to have drowned the Batateiras deltas.

2.4.5 THE IPUBI FORMATION

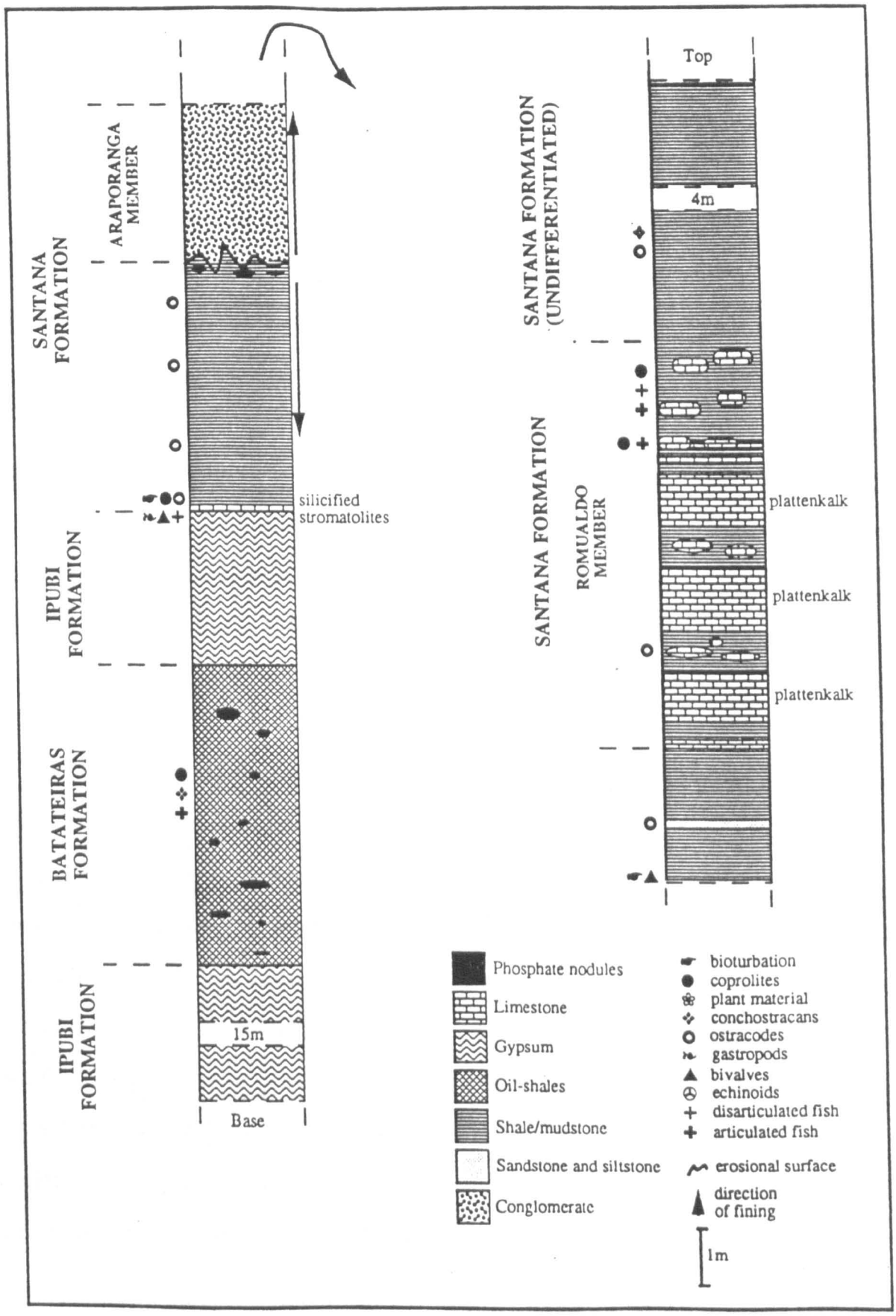
The Ipubi Formation is dominated by evaporitic facies composed predominantly of gypsum. This may be either massive, recrystallized into brown/mauve 3-4cm spherulites, or finely bedded (laminae ≈1cm thick). Its thickness varies considerably across the Chapada and is not everywhere developed (e.g. Jamacaru and Barbalha). The greatest thicknesses are at Nova Olinda (10-13m), south of Araripina (10m), and around Ipubi (20m). Silva (1983, 1986a, b) attributed variations in the development of the evaporites to a widespread post-Ipubi erosional event. However, in a re-examination of some of the localities discussed by Silva (op. cit.), I failed to find any evidence of a palaeokarst or of calcretes. The variations in the development of the evaporites across the basin (summarized in text fig. 2.11) therefore probably reflect the relative position of structural highs and lows.



Text figure 2.11: Lateral variations in the thickness of development of the Ipubi Formation, Batateiras Formation, and various members of the Santana Formation. The variations in thickness probably correspond to structural features and proximity to the Batateiras delta systems in the north-east and east.



Text figure 2.12: Sedimentological log of locality 6, gypsum quarry 15km south of Nova Olinda.



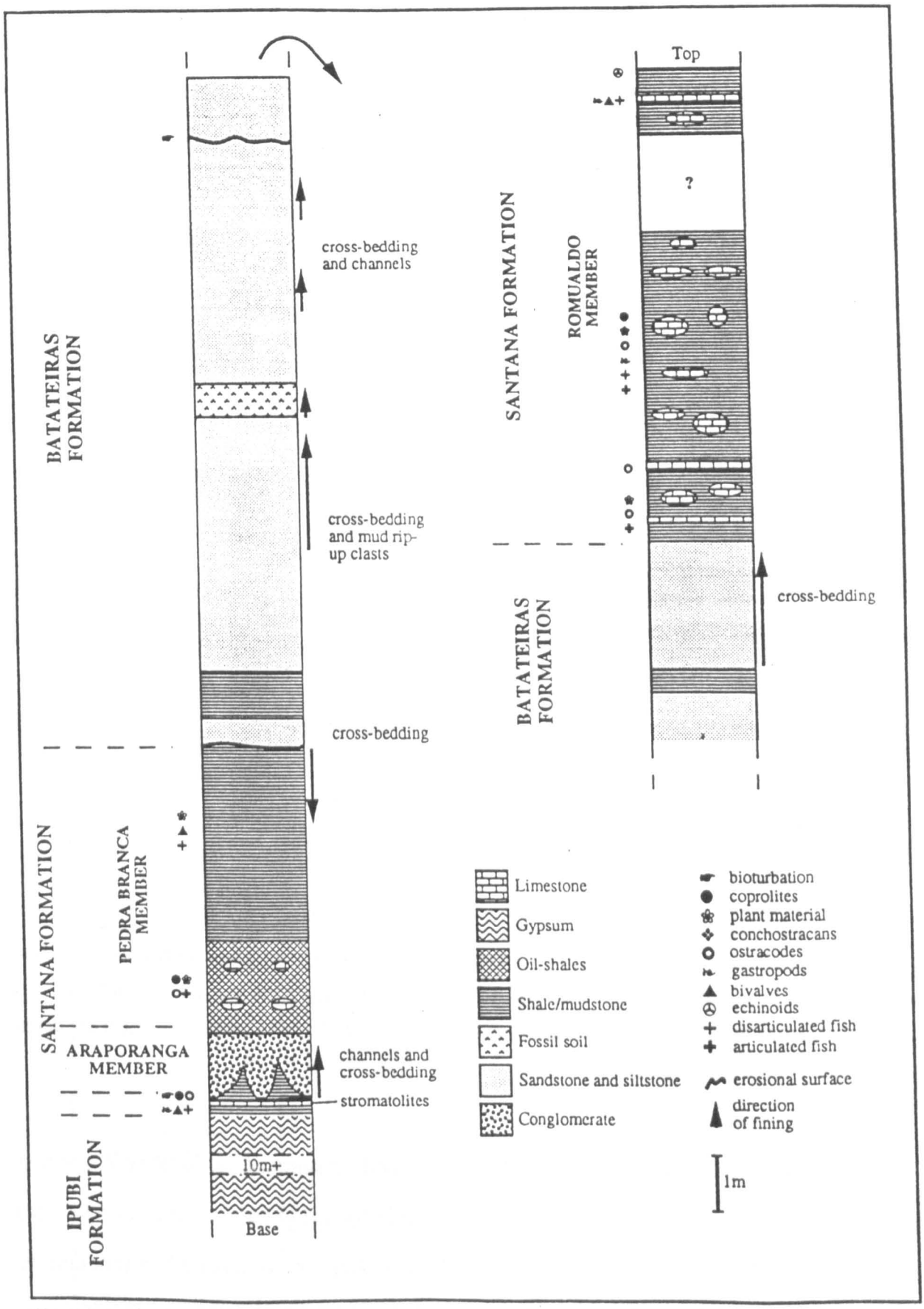
Text figure 2.13: Sedimentological log of locality 7, Mina Lagoa de Dentro, Araripina.

Silva (1983, 1986a, b) considered the evaporites of the Ipubi Formation to have formed in non-marine sabkhas. However, sedimentological evidence for a sabkha setting is lacking, and there are distinct marine signatures (Lima, 1978b; Baudin *et al.*, 1990; Berthou and Pierre, 1990). A more plausible setting would be a barred-basin. This is consistent with the structural characteristic of the Araripe Basin (see Section 2.2).

The gypsum is divided into two at several localities by clastic sediments probably referable to the Batateiras Formation. The volume of fresh water and sediment flushed into the 'Ipubi Lagoon' by these deltas was evidently sufficient to prevent the precipitation of evaporites. Near Santana do Cariri (locality 6, text fig. 2.12), the gypsum is divided into two by up to 16m of unfossiliferous, greasy green/grey shales and marls containing thin stromatolites; and at Jamacaru such sediments entirely replace the evaporitic facies. These sediments are deltaic in origin and are extremely localized in occurrence. At Mina Lagoa de Dentro (locality 7, see text fig. 2.13), ostracodes and conchostracans occur in abundance in a highly fissile oil-shale within the evaporites which probably represent an isolated, ephemeral, still water pool in which hospitable freshwater conditions were temporarily maintained by small feeder streams.

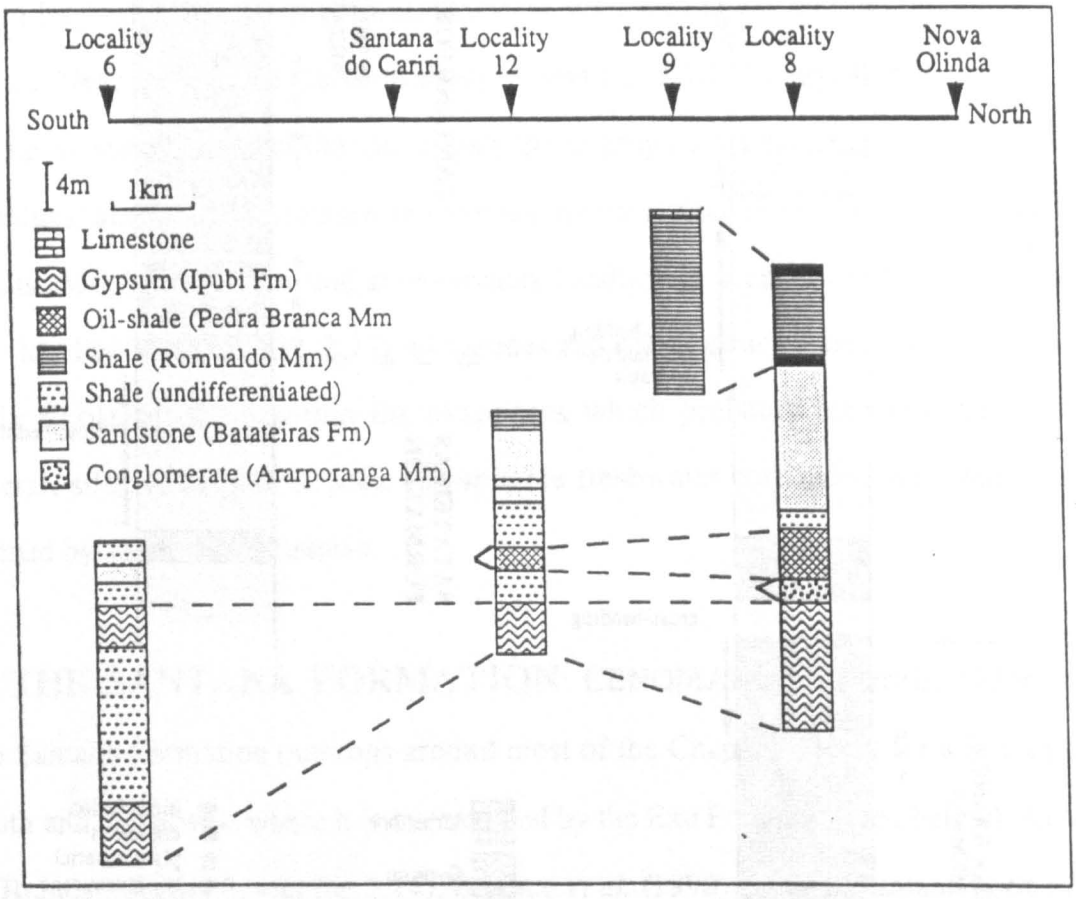
2.4.6 THE SANTANA FORMATION: CENOMANIAN (Martill, 1990b).

The Santana Formation outcrops around most of the Chapada except for a few areas in the south and north-west where it is overstepped by the Exu Formation (see below). At Mina Pedra Branca (locality 8, text fig. 2.14), Berthou *et al.* (1990) gave the Santana Formation a thickness of ≈33m. In this region (i.e. the north and east of the Chapada), the Santana Formation is lithologically diverse and three distinct members may be recognised - the Araporanga, the Pedra Branca, and the Romualdo (Martill and Wilby, 1993b). These are separated from one another by grey/green silty clays and sandstones referable to the Batateiras Formation. In contrast, around Ipubi and Araripina, the sequence is relatively monotonous and the Araporanga and Pedra Branca Members are absent or much reduced in thickness.



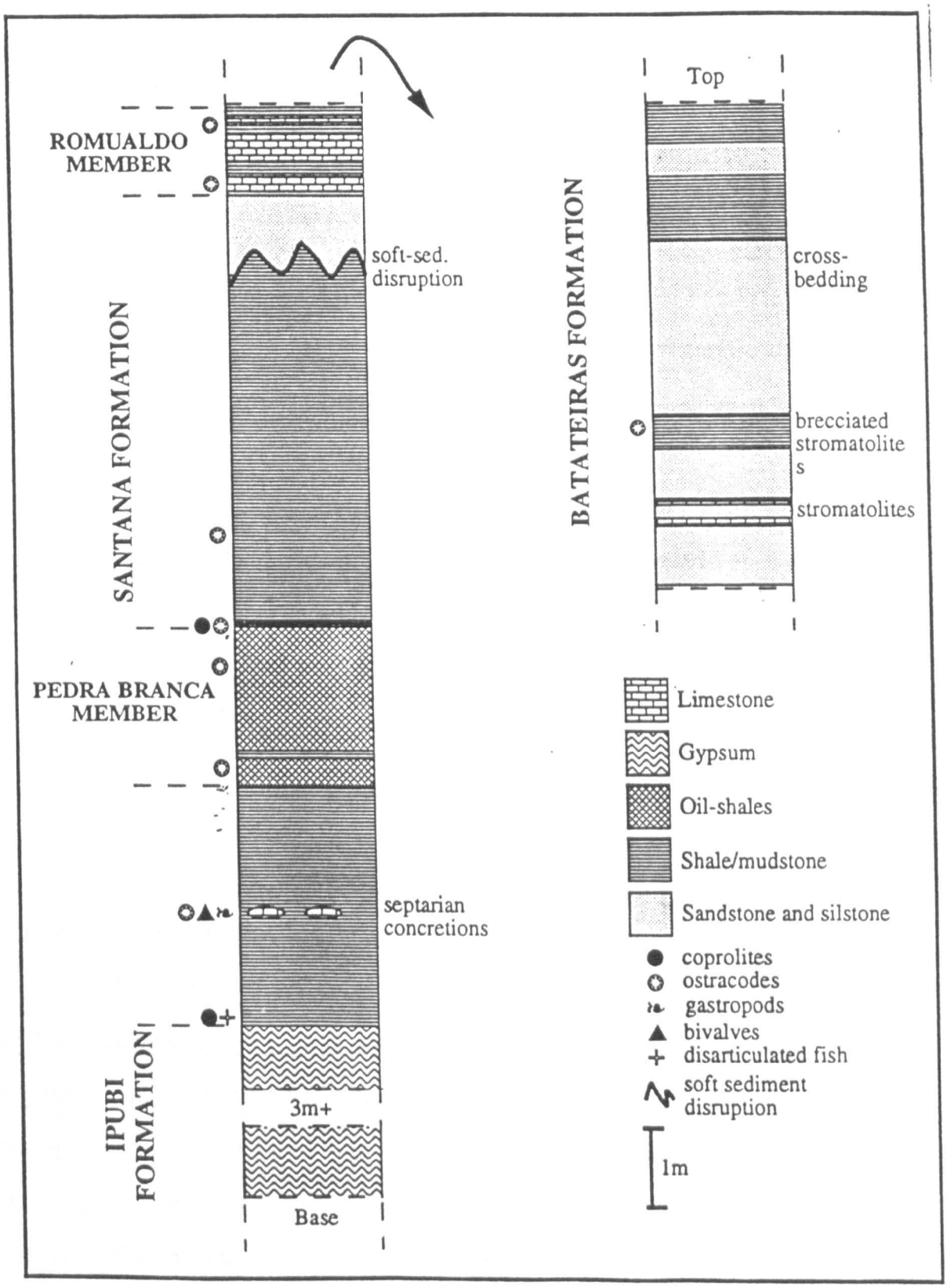
Text figure 2.14: Sedimentological log of locality 8, Mina Pedra Branca, Nova Olinda.

Interdigitated with the Santana Formation are thick units of white, cross-bedded sandstones and siltstones. These are of deltaic origin and are referable to the Batateiras Formation. In the Nova Olinda/Santana do Cariri region, variations in the extent to which these sediments are developed suggests the deltas to have advanced from the north-east (text fig. 2.15). These sandstones reach their greatest thickness at Mina Pedra Branca (locality 8, see text fig. 2.14) where over 16m occur. In the Araripina and Ipubi regions, the Batateiras deltas were not important.



Text figure 2.15: Variations in the extent of development of the Ipubi Formation, Batateiras Formation, Araporanga Member, Pedra Branca Member, and Romualdo Member in a north to south section from Nova Olinda to Santana do Cariri. Increased thicknesses of the sandstones to the north suggest the Batateiras deltas to have advanced from this direction.

The base of the Santana Formation is defined around Araripina (e.g. locality 7, see text fig. 2.13) and Santana do Cariri (e.g. locality 8, see text fig. 2.14) by numerous thin (<1cm) stromatolites and hard grounds, 'lags' of silicified ostracodes, and/or a bone bed (containing abundant hybodont and simple cone teeth, articulated fragments of an aspidorhynchiform, phosphatized coprolites, and phosphatic internal moulds of gastropods). Around Ipubi, 'greasy' green mudstones occupy the same position.



Text figure 2.16: Sedimentological log of locality 9, an old gypsum quarry 3km south of Mina Pedra Branca.

2.4.6.1 THE ARAPORANGA MEMBER

The Araporanga Member (Martill and Wilby, 1993b; equivalent to "bed PBM2" of Berthou *et al.*, 1990) occurs (see text figure 2.11) only in the Nova Olinda and Araripina regions. It lies directly on the basal undifferentiated sediments of the Santana Formation (see Section 2.4.6)(see localities 7 and 8, text figs. 2.13 and 2.14 respectively). The Araporanga Member consists of 2-4m of a light coloured, upward fining, poorly sorted conglomerate containing channels, scours and thin laterally impersistent sandstone and siltstone lenses. Its base is marked by impressive flame, and ball and pillow structures. The clasts (up to 10cm) are angular and matrix supported and include (in order of abundance) quartzite, shale, laminated limestones with ostracodes, gypsum, and rolled and bored internal moulds of gastropods; all derived from local sources.

The Araporanga Member has been widely interpreted as a mass flow deposit (Berthou *et al.*, 1990; Medeiros, 1990; Martill and Wilby, 1993b) which was dumped in a single event. Martill (pers. comm., 1990) proposed the bed to have been deposited by a sudden and catastrophic influx of marine water. However, this appears unlikely since the deposit is only locally developed and a marine fauna was already established in the Araripe Basin prior to its deposition (i.e. the bone bed immediately overlying the Ipubi Formation). Instead, the lithology probably represents a series of debris flows caused by the destabilization of talus on local highlands by earth tremors and/or torrential rains.

2.4.6.2 THE PEDRA BRANCA MEMBER

The Pedra Branca Member occurs only in the vicinity of Nova Olinda (e.g. localities 8 and 9, see text figs. 2.14 and 2.16) and rests directly upon the Araporanga Member (Martill and Wilby, 1993b). This distinctive lithology (Berthou *et al.*, 1990; Cavalcanti and Vianna, 1990) consists of ≈6m of black, organic-rich (up to 45%TOC, Baudin *et al.*, 1990), pyritic paper shales containing pelletal phosphatic lags and small (<6cm) syncompactional phosphatic and carbonate concretions. Plant debris, articulated fish including *Vinctifer*, *Rhacolepis* and *Cladocyclus* (Sales Viana *et al.*, 1989), and crushed ostracodes (exclusively *Hourcqia angulata angulata*, Krömmelbien and Weber) occur in abundance. Articulated,

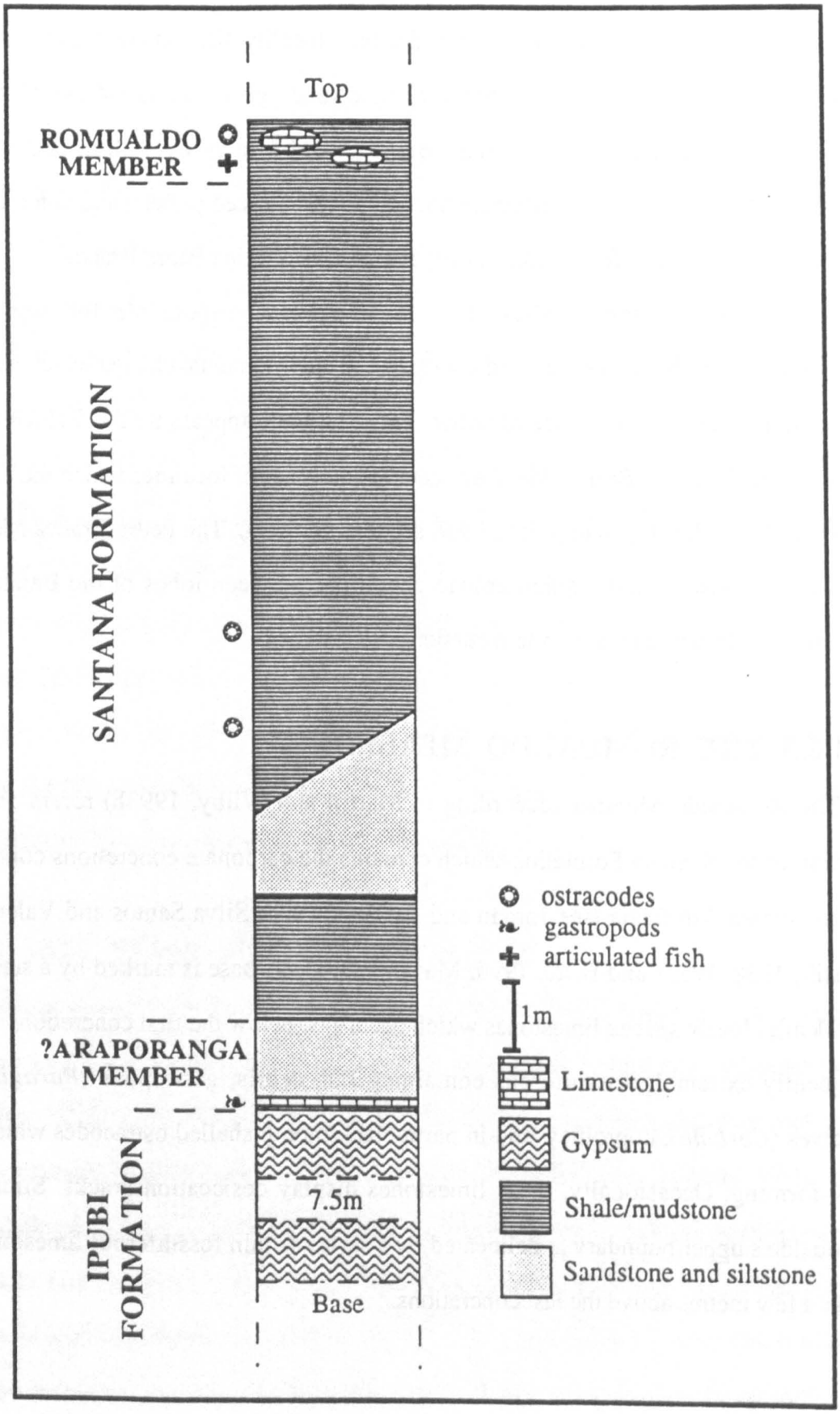
three-dimensionally preserved specimens of *H.angulata angulata* are also common in the calcite concretions.

In the Araripina region, the same stratigraphical position is occupied by monotonous mudstones containing occasional ostracodes (e.g. locality 10, text fig. 2.17). Around Ipubi (e.g. locality 11, text fig. 2.18), red silty mudstones give way to ≈4-5m of grey fissile shales at a comparable level to the Pedra Branca Member in the east. These shales do contain abundant ostracodes, small carbonate nodules, phosphatized pellets, and some fish debris, but are certainly not as fossiliferous or organic-rich as at Mina Pedra Branca.

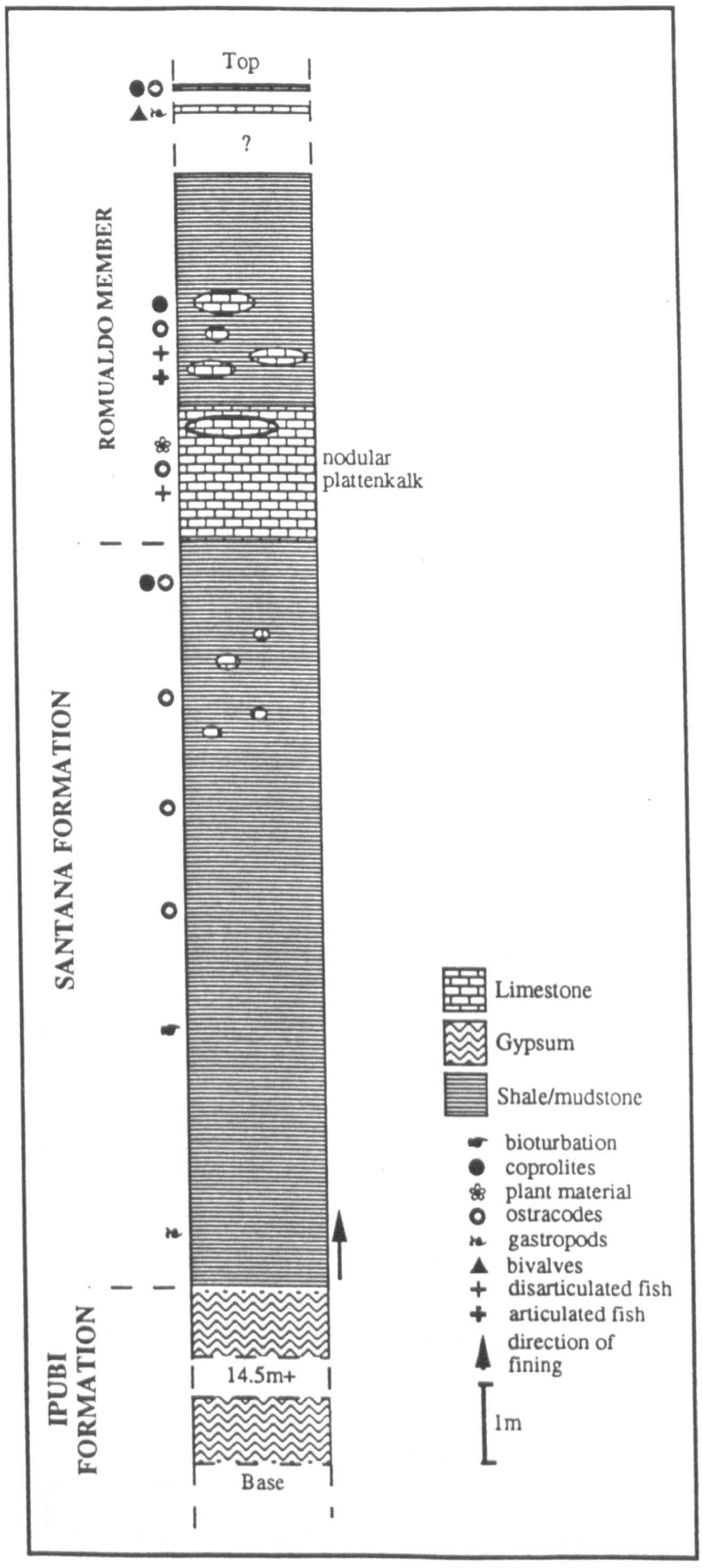
Martill (pers. comm., 1992) considers the event responsible for depositing the Araporanga Member to have caused a widespread environmental change which triggered the deposition of the Pedra Branca Member. This however, appears unlikely since lithologies referable to the Pedra Branca Member occur at a number of localities where the Araporanga Member is not developed (e.g. locality 9, see text fig. 2.16). The Pedra Branca Member was probably deposited under quiet, anoxic conditions between lobes of the Batateiras delta which were drowned by a marine incursion.

2.4.6.3 THE ROMUALDO MEMBER

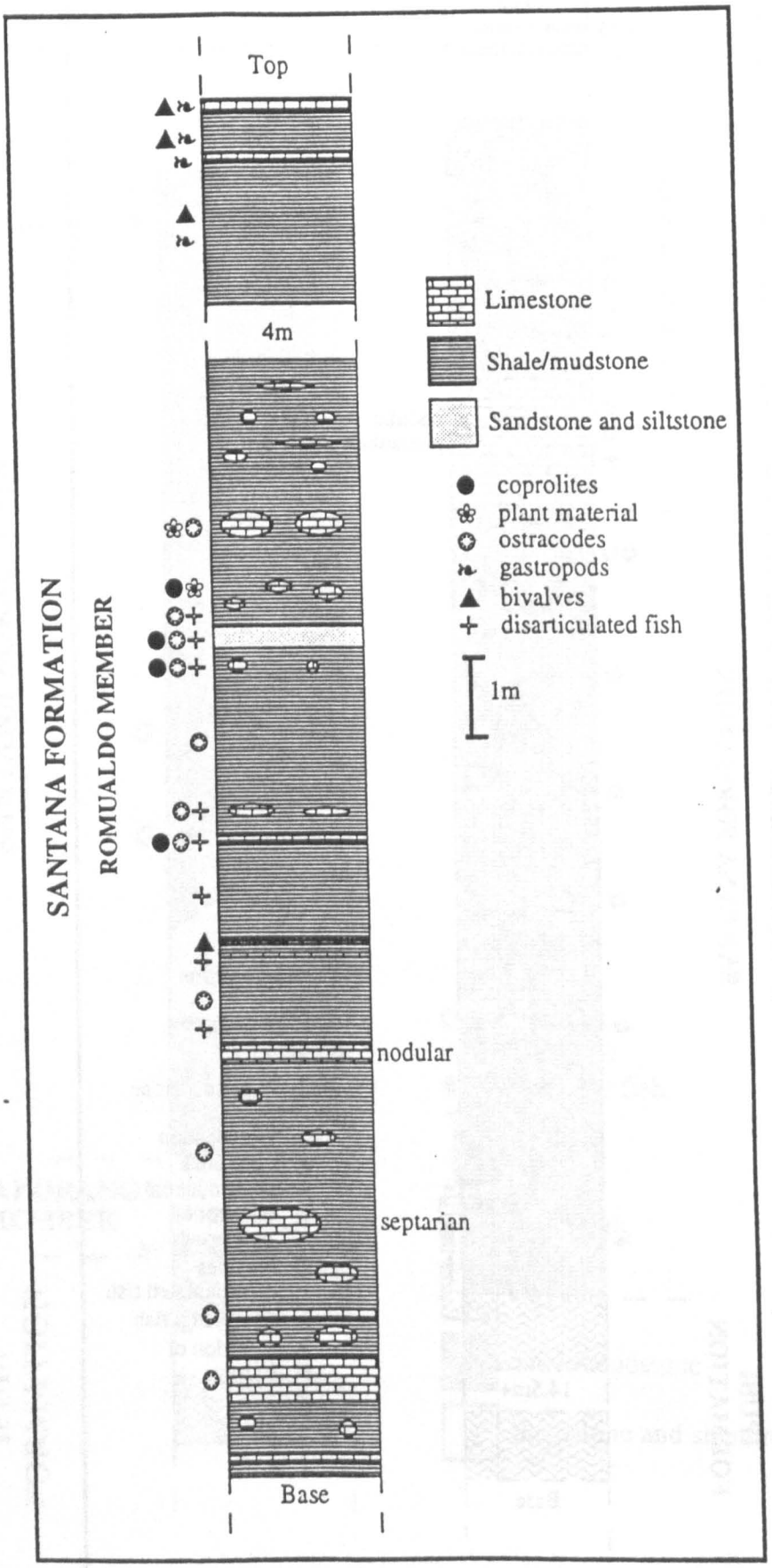
The Romualdo Member (according to Martill and Wilby, 1993b) refers only to that portion of the Santana Formation which contains the carbonate concretions containing the much studied fish fauna (see Jordan and Branner, 1908; Silva Santos and Valenca, 1968; Martill, 1988; Wenz and Brito, 1990; Maisey, 1991). Its base is marked by a series of thin (5-50cm), closely spaced limestones which occur just below the first concretions. These are frequently extremely fossiliferous containing fish debris, gastropods (*Paraglauconia*), bivalves (*Corbula*), coprolites, and in particular, smooth shelled ostracodes which may be rock-forming. Occasionally, these limestones display desiccation cracks. Similarly, the Romualdo's upper boundary is delineated by a series of thin fossiliferous limestones which occur a few metres above the last concretions.



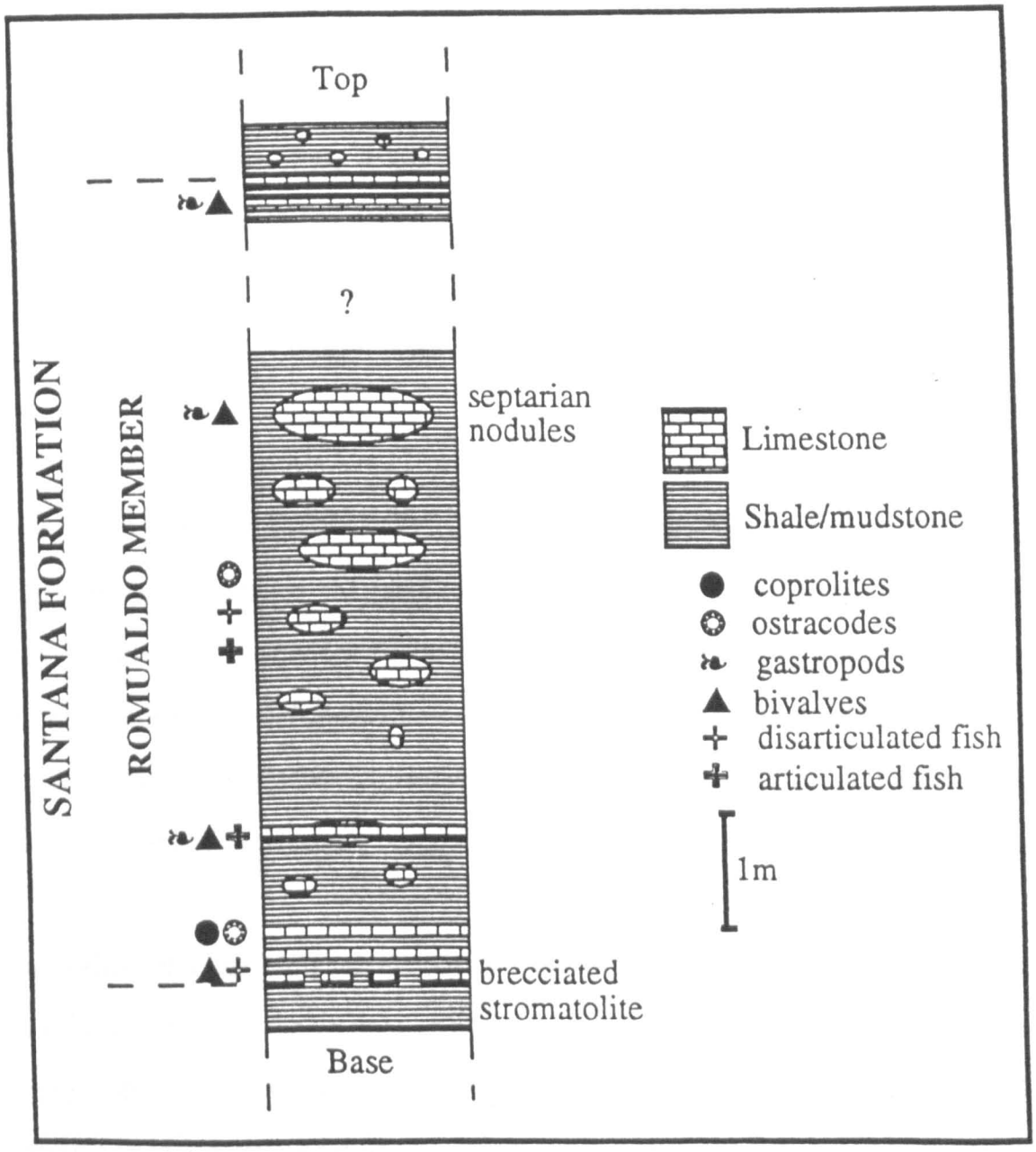
Text figure 2.17: Sedimentological log of locality 10, Mina Buracao, Araripina.



Text figure 2.18: Sedimentological log of locality 11, Alto Bonito, Ipubi.



Text figure 2.19: Sedimentological log of locality 12, stream cutting 1.5km south of Mina Pedra Branca.



Text figure 2.20: Sedimentological log of locality 13, stream cutting 3km SW of Jardim.

The Romualdo Member outcrops around the majority of the flanks of the Chapada except for in the north east where it is overstepped by the Exu Formation. Particularly good sections occur in streams and mines at Porteiras (locality 3, see text fig. 2.8), between Nova Olinda and Santana do Cariri (e.g. localities 8 and 12, see text figs. 2.14 and 2.19 respectively), Jardim (locality 13, text fig. 2.20), and Sierra do Maozina (locality 2, see text fig. 2.7). In the south and south west of the Chapada, although the upper half of the Romualdo Member is usually absent, good sections occur in gypsum quarries.

The Romualdo Member varies considerably in thickness across the basin (see text fig. 2.11). The greatest developments are to the north and east; on Sierra do Maozina (locality 2, see text fig. 2.7) approximately 30m occurs, whereas near Ipubi (e.g. locality 11, text figs. 2.18) and at Mina Lagoa de Dentro (locality 7, see text fig. 2.13) thicknesses of ≈7m are typical. The Romualdo Member is everywhere dominated by green/grey, laminated, silty shales. The shale rarely contains evidence of bioturbation, but at certain horizons, it is rich in ostracodes (*Pattersoncypris micropapillosa* Bate), plant fragments, bivalves and gastropods. Throughout the sequence, thin limestones (often ostracode rich) and stromatolites occasionally occur.

Concretions containing the exquisitely preserved fish fauna are restricted to seven or more specific horizons or zones within the member. The concretions vary greatly in character from small (<5cm) spheres, to thin lenticular bodies and oval masses up to 2m in length (but generally ≈30cm in length). It is the latter which most frequently contain the diverse and abundant fish fauna (see Section 2.5.2 for a more detailed description of their lithological, diagenetic, and faunal characteristics). Towards the top of the Romualdo Member, more massive (often over 1m diameter) dark grey, septarian concretions with a much less abundant ichthyofauna occur.

To the south (around Ipubi) and south west (around Araripina) of the basin, the Romualdo Member is condensed. Its lower boundary is defined by platy limestones which contain plant fragments, ostracodes, coprolites, conchostracans, and some fish debris (e.g. locality 11, see text fig. 2.18). Individual limestones may be up to 2m thick, but more commonly, several thinner units are separated by concretionary shales. These limestones give way to rarely more than 3m of grey/green shales containing lenticular concretions

typical of the Romualdo Member. Above, up to 8m of monotonous shales occur in which conchostracans may be locally abundant.

Above the Romualdo Member, across the entire basin, the Santana Formation continues for several metres. These sediments are poorly exposed and have yet to be formally described, although they have been logged at Mina Pedra Branca (locality 8, see text fig. 2.14) by Berthou *et al.* (1990a). They are particularly well exposed at Sierra do Maozina (locality 2, see text fig. 2.7) where they consist of highly fossiliferous, light grey and brown silty shales with numerous thin bioclastic limestones. A variety of small bivalves and in particular the gastropod - *Paraglauconia* cf. *lyrica* - occur in such abundance as to be rock forming (see Martill and Wilby, 1993b, text fig. 3.16). Worn fish debris and poorly preserved echinoderms (attributed to *Faujasia* and *Pygurus*, see Beurlen, 1966) occur not only in the SW (Beurlen, 1963) but also at Mina Pedra Branca (locality 8) and Porteiras (locality 2). In places, these limestones are intensively burrowed. These limestones and their diagenesis are described in detail by Berthou *et al.* (1990, Plate 5, figs. 1-3, 5; Plate 6, figs. 1-4).

2.4.7 THE EXU FORMATION: ALBIAN (Lima, 1978c, 1979b) or EARLY CENOMANIAN (Berthou, 1990).

The Exu Formation dominates the profile of the Chapada do Araripe forming a prominent cap along the Chapada's entire length. It consists of 250-300m (Beurlen, 1962; Ponte and Appi, 1990) of massive-, planar- and cross-bedded, red, yellow, and ochre conglomerates, sandstones and siltstones (Mabesoone and Tinoco, 1973; Ponte and Appi, 1990; Martill and Wilby, 1993b). The coarser and more generally massive sediments are typical of flash flood deposits, whilst the stratigraphically more important finer grained planar- and cross-bedded sediments were probably deposited by meandering and braided (especially those consisting of repeated fining-up packages) river systems. Fossils, except for spores and pollen (Lima, 1978c, 1979b) are extremely rare.

2.5 THE CONCRETIONS OF THE ROMUALDO MEMBER

The concretions of the Romualdo Member vary enormously in size and shape from small (<2cm) disc-like bodies without any obvious organic nucleus, through ovoid and disc-shaped masses averaging 30cm in length with a well preserved vertebrate fauna, to massive (often >1m diameter) septarian concretions containing imbricated accumulations of bivalves and scattered fish material. These come from different horizons within the Romualdo Member (see Section 2.4.6.3). Only the ones containing the exceptionally preserved vertebrate fauna are dealt with in detail here.

Martill (1988, text fig. 3) categorized the fossiliferous concretions of the Romualdo Member according to their shape, size, and manner with which they enclose the fossil remains, whilst Maisey (1991, pp59-66) divided them into three types based predominantly on their fossil content, and lithology. Maisey's (1991) scheme must be treated with some caution. Examination of thousands of concretions in the field and a detailed petrographical investigation of his three concretionary types indicates that one - the "Jardim-type" (Maisey, 1991, p62) - is merely a weathered form of another - the "Old Mission-type" (Maisey, 1991, p63). Furthermore, the naming of concretionary types after localities is somewhat misleading. Although Maisey's "Santana-type" concretions are restricted to the area immediately around Santana do Cariri, "Old Mission-type" concretions occur around the entire basin. These two types of concretion are therefore referred to in this thesis as 'type 1' and 'type 2' concretions respectively. The characteristics of these two types of concretions are described separately below:

a) Type 1 concretions (or Santana): These are ovoid, rather platy wackestone/packstone concretions up to 30cm in maximum dimension, which are usually flattened in the plane of the sediment. Type 1 concretions occur only at the base of the Romualdo Member, and are restricted to the region surrounding Nova Olinda. Their shape does not reflect the outline of the enclosed fossil(s) (see fig. 2.28). Laminae are widely spaced and composed predominantly of articulated ostracode tests. Pyrite and argillaceous material is rare.

2) Type 2 concretions (or Old Mission): These are ovoid, well laminated concretions, whose outline often accurately reflects that of the enclosed fossil (see fig.

2.25). They often exceed 30cm in maximum dimension, and occur at specific horizons across the entire outcrop of the Romualdo Member. When unweathered, they are a distinctive blue/grey colour, whereas weathered examples are buff/cream. Frequently, there is a gradual transition in colour from the core of the concretion (blue/grey) to the mantle (cream). Individual laminae are usually less than 1mm thick (a minimum of 50μm, Berthou *et al.*, 1990, p177) but may be up to 5mm thick. These consist of alternating light (carbonate-rich) and dark (organic-rich) bands which are laterally persistent (fig. 2.5). The carbonate-rich laminae are composed of microspar which appears to have grown non-displacively in the sediment's original pore-spaces. Acid digestions of type 2 concretions suggest a precompactional porosity of the sediment of 85-92%. This is comparable to porosities recorded from early diagenetic concretions elsewhere (see Raiswell, 1971, 1976; Hudson, 1978, pp346-347).

Many of the organic-rich laminae of type 2 concretions are composed of well preserved 'microbial mats' (see Berthou *et al.*, 1990, Plate 2, figs. 5-7, Plate 3, figs. 3, 4, 6). These may be extracted intact from the concretions by acid digestion, and closely resemble modern bacterial scums in appearance and texture. Indeed, Martill (1988, p14) reports having observed poorly preserved filaments in these mats. Many of the other dark laminae may have had a similar origin but are too extensively degraded to be recognised as such. Some dark laminae however, are certainly not microbial in origin, consisting instead of compacted faecal pellets. Uncompacted faecal pellets occur in abundance as geopetal fills in the protective spaces of gastropod whorls (see Berthou *et al.*, 1990, Plate 5, fig. 4) and occasionally fish carcasses.

Occasionally there is a gradational change (over 1-1.5cm) in the thickness of the two types of laminae, so that the concretion becomes progressively more carbonate-rich upwards before returning sharply to a thick organic-rich lamina (see fig. 2.5). It is not clear whether this grading represents seasonal fluctuations, the switching of sediment sources, gravity settling of mobilized sediment, or indeed some other cyclic or repetitive environmental parameter.

Vertebrate fossils are extremely abundant and well preserved in type 2 concretions. Frequently, more than one fish is preserved on the same lamina. Invertebrates in contrast,

are rare except for ostracodes which are dispersed either evenly throughout the concretions, or are concentrated at certain levels.

Several type 2 concretions examined contained scours in which imbricated and crudely graded ostracodes, coprolites, fish debris, well preserved plant material, articulated bivalves, phosphatized muscle, and gastropods (in that order of abundance) occur (figs 2.6 and 2.7). Some of these 'event' horizons cover areas in excess of $0.3m^2$ but rarely occupy hollows of more than a few centimetres depth (see Section 2.5.4. for a discussion of their genesis). Compaction of some of the coprolites indicates not all of the faeces to have been mineralized prior to incorporation into the scours.

2.5.1 DIAGENESIS OF THE ROMUALDO MEMBER CONCRETIONS (see text fig. 2.21 for a summary).

The Romualdo Member concretions have an extremely complex diagenetic history dominated by the precipitation of non-ferroan calcites. Some fish within the concretions contain over ten generations of concentric fringing calcites (fig. 2.8) as well as various late stage accessory minerals such as gypsum, celestine, and barytes. Disseminated, framboidal, and 'mouldic' (i.e. occurring only in specific microenvironments such as gastropod shells) pyrite is also common. Unfortunately, no comprehensive diagenetic investigation (including isotopic analyses) of these cements has been performed. This is certainly beyond the scope of this study; the following comments are therefore restricted to a general description of the typical sequence of cements, and a discussion of those diagenetic models which have been postulated. Emphasis is placed on the diagenetic history of the type 2 concretions.

Both the type 1 and type 2 concretions are most commonly cemented by non-ferroan microspar. Most originally aragonitic shelly material (e.g. gastropods) is replaced by drusy calcite, and their tests either filled with spar or micritic sediment. In contrast, although most calcitic tests (e.g. bivalves and ostracodes) have been neomorphosed, they usually retain relics of their original structure.

Occasionally, type 2 concretions are composed of a chalky, ferroan calcite (fig. 2.9) broadly similar in texture to adipocere recorded replacing Recent fish from Lake Onondaga,

U.S.A. (Dence, 1956; Sondheimer *et al.*, 1965; Wilcox and Effler, 1981)[1] . The chalky type 2 concretions are themselves enclosed by non-ferroan calcite mantles more characteristic of other calcitic concretions. Disruption caused by the collapse of the fish carcasses indicates the chalky concretions to have formed prior to the release of putrifaction gases, and therefore to have developed within a matter of weeks of the fish's death. Such a timing is consistent with the rate of development of adipocere concretions (Berner, 1968a; Breder, 1957, p133; my own actualistic experiments) but not with that of even small calcite concretions (Berner, 1968b estimates an 'average' calcium carbonate concretion of 1cm radius to take ≈500yrs to develop). Some other concretions, although not displaying textures reminiscent of adipocere, do contain evidence of 'brittle' fracture above the carcasses (fig. 2.10). This implies the sediment to have been at least partially mineralized prior to the organism's collapse. However, most concretions display only soft sediment disruption indicating the mineralization of *most* to have post-dated the collapse of the fish.

Mabesoone and Tinoco (1973, p106) considered all type 2 concretions to have formed around fish which had been washed up on to muddy beaches. They (Mabesoone and Tinoco, op. cit.) postulated that when exposed to strong solar rays, organic fats (predominantly lipids) released from the carcasses had cemented the surrounding sediment to form the concretion. However, subaereal conditions are not consistent with palaeoenvironmental (see Section 2.5.4) and taphonomic (see Section 2.5.3) evidence. This model has therefore not received widespread acceptance.

The space formerly occupied by soft tissues of fish from type 2 concretions is either 1) infilled with sediment invaginations (fig. 2.11); 2) entirely filled with 2-20^{+} centripetal generations of calcite (see fig. 2.8); or, 3) remains largely hollow (fig. 2.12). In all cases, the very first calcite phase is a light brown, isopachous, fibrous, non-ferroan calcite <1mm thick. This is then followed by one or more discontinuous light brown calcites, each separated by dark brown sparry calcite (fig. 2.13). In fish remaining largely hollow (see fig. 2.12), these are followed by a single generation of subhedral calcite crystals and

[1] Adipocere is a calcium fatty acid comonly formed in and around decomposing carcasses (see Berner, 1968a). It is believed by many workers (Berner, 1968a; Criss *et al.*, 1988) to be a precursor of $CaCO_3$ in calcitic concretions.

occasionally by yellow calcite rhombs and/or accessory minerals. In fish containing a greater quantity of diagenetic calcite, these fringing concentric cements continue to develop until the cavities are entirely filled. Fish fossils midway between these two extremes (i.e. small unmineralized cavities still exist within their body cavities, fig. 2.14) are common.

In type 1 concretions, only late stage yellow and clear sparry calcites are well developed, although occasionally a dark brown calcite occupies the former position of the kidneys.

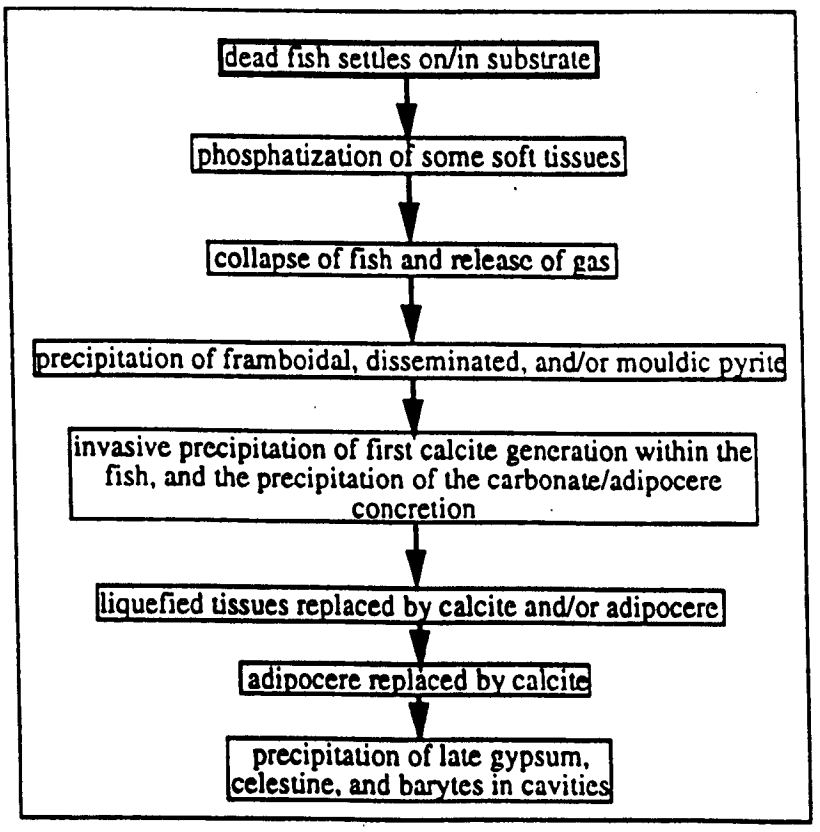
The timing and genesis of these calcites is problematical but certainly *most* occurred after the phosphatization of the soft tissues (see Chapter 6). Mabesoone and Tinoco (1973, p106) considered the soft parts of each fish to have first been "cooked" by solar rays and then to have been destroyed by plasmosis. This created a hollow internal mould in to which calcite was subsequently precipitated. On top of sedimentological and taphonomical objections (see above), this model is also contradicted by the timing of growth of *most* of the carbonate concretions relative to the *first* calcites fringing the inside of the carcasses. Mabesoone and Tinoco (1973, p106) proposed the concretions to have developed some time before the fish themselves were mineralized. However, the presence of soft sediment collapse structures above *most* of the fish (see fig. 2.8) indicates the concretions not to have developed rapidly enough to have maintained the fishes' three-dimensional shapes (as proposed by Mabesoone and Tinoco, 1973). Instead, their morphologies were probably maintained prior to the growth of the concretion by extremely early calcites within the carcasses themselves. Indeed, Maisey (1991, pp78-80) proposed the first calcites inside the fish to have been so early as to have preceded any visible signs of decay, and to have occurred whilst the fish were 'bobbing' above the sediment surface. Although the first calcites were extremely early, this scenario is inaccurate. All specimens examined indicate the first calcites within the fish to have developed after ruptures in the body walls of the fish; the calcites form a continuous fringe around and over ruptured sections of the fish (see fig. 2.8).

It is not clear how space was made available for these early cements. There is some evidence to suggest they grew invasively into 'liquefied' tissues. For example, the ultrastructure of some portions of phosphatized dermis is disrupted by euhedral calcites (fig. 2.15). These appear to have grown into the tissues before or during phosphatization. Such observations are consistent with Briggs and Kear's (1993a) experiments which

demonstrated that calcites may grow in decomposing organisms extremely rapidly after death.

Whilst the first calcite phases appear to have been precipitated pre- or at the latest syn-concretion, the later calcites (which occupy the majority of space in most fish from type 2 concretions, see figs. 2.8, 2.13, 2.14) seem to have grown in to a liquid or gas filled void some time after the collapse of the carcasses. The presence of gas cavities in the upper half of fish, and the accumulation of skeletal debris and phosphatized soft tissues in the base of many fish (see fig. 2.8), suggests those soft tissues which had not been phosphatized, were at this point greatly decayed. The mutual separation of this debris and the occurrence of 'floating' skeletal elements implies them to have settled on and in liquefied soft tissues which were subsequently calcified. Without further investigation, it is impossible to decide whether this liquefied tissue was first replaced by adipocere, or was replaced directly by calcite.

Whatever the process(es) involved in the growth of these carbonate concretions and the precipitation of calcite within the carcasses, it is clear that the speed at which it took place was crucial to the preservation of the enclosed fossils' three-dimensional morphology. Concretion growth clearly pre-dated the most intense phase of compaction.



Text figure 2.21: A theoretical diagenetic profile for the type 2 concretions of the Romualdo Member.

2.5.2 THE FAUNA AND FLORA OF THE ROMUALDO MEMBER

The palaeontology of the Romualdo Member has recently been reviewed in detail by Maisey (1991) and Martill (1993a). The macrofauna is dominated by a diverse assemblage of vertebrates, and in particular by actinopterygian and elasmobranch fish (see Wenz and Brito, 1990; Maisey, 1991; Wenz *et al.*, 1993). At least 19 species of 16 families occur (Wenz and Brito, 1990). These are preserved in exceptional three-dimensional detail. Turtles (Price, 1973), crocodiles (Price, 1959; Kellner, 1987), pterosaurs (Price, 1971; Kellner, 1989, Wellnhofer, 1985, 1991), and a dinosaur (Campos, 1985) have also been recorded.

The invertebrate fauna is much less diverse and with the exception of smooth shelled ostracodes which are locally rock forming, is relatively sparse. The ostracode fauna is dominated by *Pattersoncypris micropapillosa* (see Depeche *et al.*, 1990 for a review). Other arthropods include the earliest recorded parasitic copepod (Cressey and Patterson, 1973; Cressey and Boxshall, 1989), a crab (Martins-Neto, 1991, p432), anostracans (Maisey, 1991, p410), palaemonids (Beurlen, 1963), and a variety of unidentified shrimps (Wilby and Martill, 1992). Articulated palaemonids are common in thin platy limestones near Simões, and decapod shrimp debris (probably exuviae or coprolitic associations, see Section 4.2.3.2) is abundant in most concretions.

Mabesoone and Tinoco (1973) list a relatively sparse gastropod and bivalve fauna. With the exception of certain 'event' horizons (or scours, see Section 2.5), molluscs are fairly rare. Ammonoids are entirely absent. Beurlen (1963) also recorded echinoids from the Santana Formation but gave no indication of their stratigraphical position. These have only been recorded in the present study from the thin bioclastic limestones just above the Romualdo Member.

Dinoflagellates and foraminifera have recently been recorded from the Romualdo Member and also from just above (Arai and Coimbra, 1990; Berthou *et al.*, 1990).

The macroflora of the Romualdo Member has recently been reviewed by Crane and Maisey (1991). Although plant material is relatively abundant (see fig. 2.6) and well preserved (fig. 2.16) in the north and east of the Chapada, it is of low diversity; only

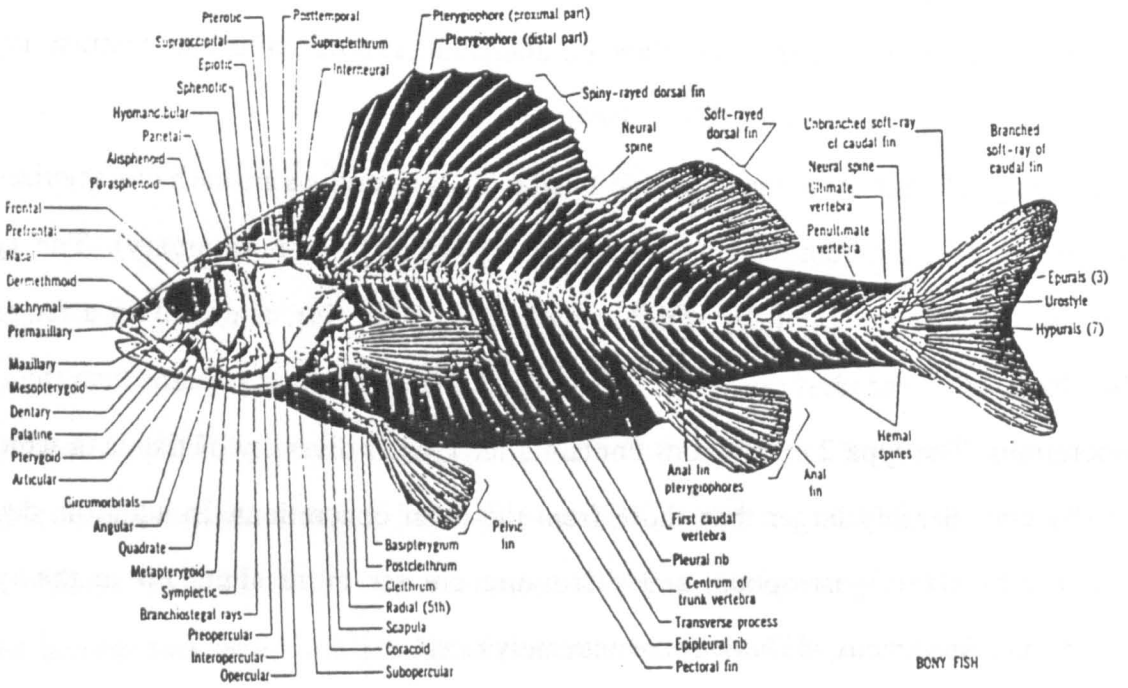
fragments and leafs of *Brachyphyllum* are abundant. The microflora in contrast, is fairly diverse (Lima, 1978a, 1978b, 1979a, 1980).

Although sharing some taxa, the type 1 and type 2 concretions are each characterized by a distinctive fossil assemblage (see Maisey, 1991, p68 for a summary). The type 1 concretions are dominated by small specimens of *Tharrias*, and contain a far greater abundance of ostracodes, crocodiles, turtles, and terrestrial plant remains than the type 2 concretions. The type 2 concretions contain a far greater diversity of fish taxa which are usually considerably larger than those from the other concretions. In addition, decapod shrimps, bivalves, gastropods, and pterosaurs are far more abundant in the type 2 concretions. Specimens of *Tharrias* are extremely rare.

2.5.3 TAPHONOMY OF THE ICHTHYOFAUNA (refer to text fig. 2.22).

Taphonomic analyses of fish have been utilized successfully by a number authors as an indirect source of data concerning the limnological conditions, biological processes, and the depositional environment of fossiliferous sediments (e.g. Hecht, 1933; Zangerl and Richardson, 1963; Schäfer, 1972; McGrew, 1975; Elder, 1985; Elder and Smith, 1984, 1988). It is therefore surprising that, despite the infuriating lack of diagnostic taxa in the Romualdo Member, the fish of the Romualdo Member have not been subjected to a taphonomic investigation (with the exception of the short and somewhat confused examination presented by Maisey, 1991). Presented below is a review of the most palaeoenvironmentally informative taphonomic manifestations encountered in this study:

Based on taphonomic criteria, fish remains in the concretions of the Romualdo Member may be divided into 2 broad categories. These are: a) ubiquitous disarticulated debris scattered throughout the sediment indicative of the disarticulation of floating carcasses (Schäfer, 1972, p58; Elder, 1985, p31), and b) articulated or partially articulated fish. Only the latter are examined in detail here. The relevance of disarticulated fish material to constraining the temperature profile of the Romualdo Lagoon is discussed in Section 2.5.4.



Text figure 2.22: The skeletal anatomy of an actinopterygian (taken from Lagler *et al.*, 1962).

A discussion of the taphonomy of all of the taxa thus far recorded (see Maisey, 1991) is impracticable, especially since some species are extremely rare. Detailed descriptions are therefore restricted to the 4 commonest fish - *Rhacolepis, Notelops, Vinctifer,* and *Tharrias.* The first three taxa are from type 2 concretions; the description of *Tharrias* is based entirely on specimens from type 1 concretions.

Rhacolepis: *Rhacolepis* is the most abundant and exceptionally well preserved fish of the Romualdo Member, often occurring in large numbers as mass mortalities. In hand specimen, *Rhacolepis* usually appears three-dimensional. Sectioning however, indicates that in the majority of cases, only the specimen's 'lower' surface is intact; the upper one having either collapsed (see fig. 2.8), become distended by decay gases, or 'exploded' and disassociated (as in *Notelops*, see fig. 2.23). The head, and dorsal, anal, pelvic and pectoral fins of almost every *Rhacolepis* examined were well preserved, although the latter are very often excessively fanned. The caudal fins except for their epurals, urostyles and hypurals usually lie beyond the extent of the concretions.

Rhacolepis exhibits 2 distinctive preservational styles which suggests that they followed one of two different taphonomic pathways. In the first, the skeleton lies parallel to the bedding (i.e. they are 'bedding-plane parallel'), whilst in the second, the skeleton lies at an

angle to the bedding. The latter were termed "transgressive" by Maisey (1991, p67). The taphonomy of each is discussed separately below:

1) Bedding-plane parallel (or non-transgressive) *Rhacolepis*: Most specimens of *Rhacolepis* lying parallel to the sediment surface have sunk *at least* 1cm into the sediment; laminae directly adjacent to the body are downwarped (fig. 2.17). In many cases, although obviously bloated or disrupted by the escape of putrefaction gases, refloatation of the carcasses did not ensue. Many examples are however, entirely infilled with homogenised sediment (fig. 2.18) which entered the body cavity through ruptures in the dermis. Such specimens are usually three-dimensional and small scales on their upper surface are still largely articulated. This suggests a thin veneer of sediment to have existed above the carcass, and the escaping decay gases to have been evacuated slowly.

The vertebral column and ribs of all specimens of *Rhacolepis* lying parallel to the sediment display some disruption. Most frequently, the back-bone remains fully articulated, and is simply displaced along with any phosphatized soft tissues to the base of the carcass (see fig. 2.18).

Occasionally, small corbulid bivalves are concentrated either within the carcass or occur directly above it (see fig. 2.18). These, were probably swept into the depression created by the collapsing carcass. In one example (PRW/19), bivalves were banked up against one side of the fish, implying the carcass projected above the sediment surface during decomposition.

2) Transgressive *Rhacolepis*: According to Maisey (1991, p63), up to 50% of all three-dimensional specimens of *Rhacolepis* are transgressive. Field observations however, suggests this to be a substantial underestimate of the actual numbers; a more realistic figure being ≈80%. Most are inclined at $\approx 45^0$ to the bedding, although shallower angles are common and vertical examples have been collected. All penetrated the sediment head first and the majority (≈80%) are in a 'dorsal-up' orientation.

All transgressive fish appear to have collapsed in a remarkably similar way, and the pattern of sediment disruption surrounding the fish is essentially identical in all cases. Longitudinal sections reveal the sedimentary laminae adjacent to the fish to be homogenized in a wedge-shaped zone anterior of the skull's greatest diameter and posterior of the dorsal fin (see fig. 2.14). Along the remainder of the body, laminae are deflected downwards

around the fish without any apparent chaotic disruption. Transverse sections indicate there to be a sharp down-turning of the laminae adjacent to the fish's mid-line.

Maisey (1991, p79) proposed a rather questionable model to account for transgressive fish. In order to explain their bloated morphology and the absence of the caudal fin in many specimens, he suggested a period of floatation and decay to have occurred prior to the fish's descent to the sediment. He did not explain how the fish had sunk without experiencing any form of disintegration from the necessary expulsion of gases. Maisey (op. cit., pp78-79) implied that they had then 'bobbed' around, head-down above the substrate surface until they were buried. This model is entirely at odds with the exceptional, three-dimensional, fully articulated preservation of these fish. According to Schäfer (1972, p58), Elder (1985, p31), and my own actualistic taphonomic experiments, fish that experience floatation rapidly disarticulate. Maisey (1991, p78), proposed that these objections could be overcome and the bloated form of the carcasses maintained if pre-burial calcification took place. Although rapid mineralization is considered to be one of the most important means of preventing the loss of anatomical information (Allison, 1988a), it has traditionally been associated with the sedimentary realm rather than the aqueous one (although see Schultze, 1989).

Maisey's (1991) model also contradicts the pattern of sedimentary disruption associated with these fish. Even in a motionless water column, the continual repositioning of a bloated carcass (due to shifting pockets of decay gas) whether partially buried or 'bobbing' freely above the sediment surface, is likely to cause considerable disruption to the laminae immediately adjacent to the fish. This is not seen. The structures are more consistent with the fish having penetrated the sediment immediately after death following a rapid descent through the water column.

Rapid burial is commonly cited as an important prerequisite for exceptional preservation (e.g. Seilacher *et al.*, 1985; Brett and Seilacher, 1991). This removes the carcass from scavengers and water currents, and prevents refloatation. In the present case, instantaneous incorporation into the sediment may additionally have provided support for the carcasses during decomposition, and have given them instant access to a source of mineralizing ions.

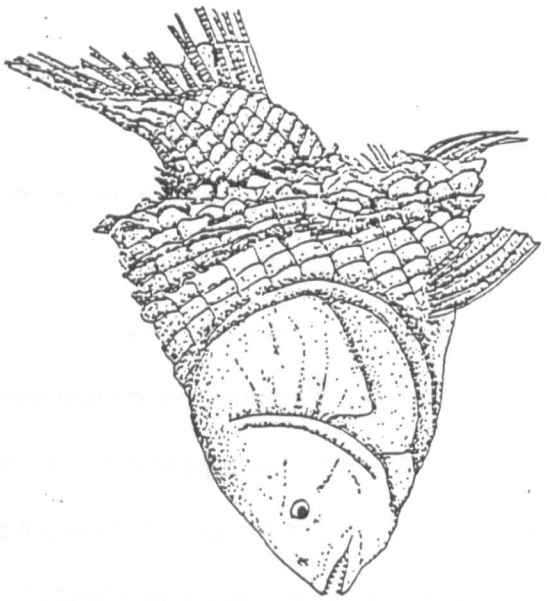
Decay followed very similar paths in all transgressive specimens of *Rhacolepis* examined. Commonly, the high angle of repose of the carcasses, coupled with a decay-

induced reduction in the competence of their tissues, prompted articulated sections of the dermis to collapse over one another down the fish's length (fig. 2.19). In response to this 'telescoping', the vertebral column in many fish displays a series of overlaps. In polished cross-sections of these fish, this is evidenced by the presence of a series of stacked sheets of scales and/or the occurrence of more than one vertebra (see fig. 2.13). In specimens of *Rhacolepis* lying normal or nearly normal to the bedding, telescoping may be intense. Often in such cases, all the vertebrae and ribs and any phosphatized musculature is geopetally concentrated in the skull; the body being completely empty (see fig. 2.12). Frequently such specimens are excessively bloated by decay gases, but remain entirely intact.

Large internal build-ups of decay gas, particularly in the body cavity prompted some 'dorsal-up' carcasses to rotate into a more 'ventral-up' position. This is attested by rotational displacement of the dorsal fin, and by the rotational collapse of the fish's body walls. Such tears result in articulated sections of the dermis from the lower surface of the fish being forced up (together with sediment) into the fish's body cavity beneath the body wall of its upper surface (fig. 2.20).

Despite the enormous quantities of gas produced during decay, very few fish appear to have shifted vertically through the sediment. In most cases, the gas was relieved by ruptures in the dorsal body wall just behind the head (see fig. 2.8) and sediment did not invade the body. Micro-faults are commonly preserved overlying such fish (see fig. 2.10). Frequently, small bones and scales are aligned in the path taken by the escaping gas (see fig. 2.9).

A number of other fish taxa also entered the substrate of the Romualdo Lagoon at various angles to the horizontal. *Lepidotes* may similarly occur both as transgressive and bedding-plane parallel fossils, although in most examples, the head is at least partially transgressive. In one particular specimen of *Lepidotes* (text fig. 2.23) which entered the sediment at a vertical angle, telescoping was so severe that the ≈40cm fish was reduced to only 15cm length, the entire body having collapsed into the skull.



Text figure 2.23: A vertical transgressive *Lepidotes* sp. in which postmortem telescoping of the body was severe and resulted in 25cm of the fish's body collapsing into its skull (based on specimen PRW/23).

Notelops: Although of very similar morphology and frequently of a comparable size to *Rhacolepis*, *Notelops* displays a very different taphonomy. It is preserved almost exclusively parallel to the bedding, rarely having sunk more than a few millimetres into the sediment. Although usually appearing three-dimensional in hand specimen, sectioning indicates that all specimens are flattened by decay-induced collapse (see Zangerl, 1971). In almost all cases, the head is three-dimensionally preserved. The fins of most are well preserved and in many are excessively fanned. This is probably a result of postmortem dehydration (see Section 2.5.4). In one case, contraction of the fin musculature was so intense as to have ripped the caudal fin in half down its centre and rotated the distal end of each half anteriorly, so that they lie parallel to the caudal peduncle (fig. 2.22).

In the majority of specimens, the upper surface of the fish has experienced severe disruption resulting from the release of decay gases. Scales and bits of fossilized soft tissue overlying the body cavity are (depending on the force of the 'explosion') either scattered in all directions beyond the carcass (fig. 2.23), or remain disorganised in the body cavity apparently having collapsed in (see fig. 2.5). In the latter case, large scales from the upper surface, phosphatized muscle, and skeletal material lie as a geopetal fill, directly on the 'hollow shell' of scales formed by the lower side of the fish (see Section 4.2.1 for a more detailed description of the disruption of the soft tissues). Frequently, large internal organs

such as the alimentary tract display signs of brittle fracture implying the soft tissues to have been phosphatized prior to the release of the putrefaction gases (see Chapter 7 for the timing of mineralization).

Both anterior and posterior of the body cavity, fossilized muscle (if present), scales, and the internal skeletal elements are usually *in situ*. Even the smallest bones of the fins, and the scales covering the anterior portion of the caudal fin remain undisturbed. This, and the pristine preservation of the lower surface of most specimens of *Notelops*, suggests these fish to have sunk directly to the sediment surface after death, and not to have experienced an extended period of floatation, refloatation, and/or scavenging. In a limited number of specimens however, some of the bones of the head, the anal and pelvic fins, many of the ribs, and most of the scales are absent (fig. 2.24), and the head is only loosely attached to the vertebral column. According to Elder (1985, p30), Schäfer (1972, pp53-54) and my own taphonomic experiments, such preservation is typical of carcasses which have experienced a period of floatation. Displacement of the pleural ribs in these specimens is consistent with the release of gas from the body cavity prior to sinking.

Some specimens of *Notelops*, especially larger ones, are partially transgressive; their heads are at a considerable angle to the sedimentary laminae whereas their bodies remain parallel. Longitudinal sections through such specimens, indicate their heads to have penetrated the sediment after a rapid head-first descent through the water column. The pattern of sediment disruption is certainly not consistent with the heavy skulls having sunk slowly into the sediment from an initially parallel repose.

Vinctifer: Most specimens of *Vinctifer* are preserved on their side, are three-dimensional (although usually the body wall on the upper surface has collapsed slightly into the body cavity), are fully articulated, and with the exception of a few partially transgressive examples (see Maisey, 1991, p65 and 81), did not sink substantially into the substrate. The area formerly occupied by skeletal muscle is very commonly infilled with sediment, and the vertebrae and other skeletal elements usually lie articulated on the lower inside surface of the fish (see fig. 2.11). Septarian cracks filled with dark brown (non-ferroan) calcite frequently develop along the inside surface of the thick scales.

Very often, *Vinctifer* (and *Cladocyclus*) displays signs of extreme contortion, the body being tightly coiled (fig. 2.25) occasionally to the extent that the vertebral column is broken. This is characteristic of severe postmortem contraction of the skeletal musclature in hypersaline environments.

In specimens which came to rest on their dorsal surface, the preopercular, opercular, subopercular and other paired bones of the skull (see text fig. 2.22) commonly fall away symmetrically (fig. 2.26). This distinctive preservational style implies that the fish did not experience refloatation. Very rarely however, there is evidence for both predation and scavenging. In one specimen (PRW/26), the head and anterior portion of the trunk is entirely absent whereas the posterior portion of the fish is fully articulated. This probably resulted from predation. In another specimen (fig. 2.27), the body is largely dismembered. Small, articulated sections of the dermal skeleton are scattered in all directions in a pattern characteristic of scavenging (Elder, 1985, pp53-72).

Other large taxa such as *Cladocyclus* and *Enneles* display a very similar preservational style to *Vinctifer*.

Tharrias: *Tharrias* displays the greatest level of disarticulation of the four taxa described here. Since other taxa such as *Cladocyclus* and *Lepidotes* preserved in the type 1 concretions also display quite extensive disarticulation, the relatively poor preservation of *Tharrias* is probably not taxonomically dictated, but rather environmentally controlled (see Section 2.5.4).

The most common form of disarticulation involves the "blowing out" in a single direction away from the carcass of the ventral fins and scales in closest proximity to the body cavity (fig. 2.28). In several such examples, the translocated pelvic fins remained fully articulated and connected to the basipterygium. This is indicative of catastrophic gas escape.

Other specimens are more extensively disarticulated. The body may be severely contorted and the back-bone severed at its mid-point. Posterior to the dorsal fin, the scales usually remain *in situ*, and the caudal fin fully articulated. Anterior of this point however, scales become less abundant, pleural ribs exposed and disorganised, and the paired fins translocated from the body. The skull in many cases is only loosely associated and

frequently is in a poor state of preservation; usually the lower jaw bones and opercular series have splayed free but remain associated. In one specimen (fig. 2.29), the lower jaw bones are arranged radially around the skull. In another, the scales on the lower surface of the carcass posterior to the dorsal fin remain articulated on the sediment surface, whilst the vertebral column and skull, paired fins and scales anterior of the dorsal fin have been 'flipped' through 180^0 so that this portion of the carcass now lies on its other side. This style of disarticulation is indicative of fish which have experienced partial floatation (Elder, 1985).

Schäfer (1972, fig. 20) and Elder (1985, p24) have both noted that the skull and tail of partially floating fish tend to rest on the sediment whilst the body is raised above. Only when sufficient gas is lost from the body cavity through a rupture in the body wall does the fish finally collapse to the sediment surface. The body and skull of partially floating fish disarticulate in a predictable manner beginning with the lower jaw bones, and proceeding with the maxillae, premaxillae, scales, opercular series, shoulder girdle, pterygoids, and eventually breakage of the skull from the vertebral column (Elder, 1985, p30). Points in this sequence are recorded in various specimens of *Tharrias* (see above).

Other specimens of *Tharrias* examined were fully articulated although somewhat flattened presumably as a result of decay (Zangerl and Richardson, 1963; Zangerl, 1971).

2.5.4 PALAEOENVIRONMENTAL INTERPRETATION OF THE ROMUALDO MEMBER

The palaeoenvironment of the Romualdo Member is discussed below in terms of 7 variables. These are: 1) sediment consistency, 2) rate of sedimentation, 3) salinity, 4) water depth, 5) current activity, 6) temperature, and 7) oxygen levels. From sedimentological, diagenetic, faunal, and taphonomic evidence, it is clear that at least two distinctive environments existed. These are represented by the type 1 and type 2 concretions. Where a difference is well defined, each concretionary type is discussed separately.

1) SEDIMENT CONSISTENCY

Taphonomic data (see Section 2.5.3) suggest the sediment cemented by type 2 concretions to have been soupy, consisting of suspended clay and organic material such as bacterial mats and faecal pellets. It was evidently sufficiently soft to permit fish of ≈20cm length to penetrate *at least* 20cm beneath the sediment surface. However, it was also sufficiently competent at these depths to prevent the refloatation of the extensively bloated carcasses, and to preserve their distended morphology. Martill (1988) has suggested that this was afforded by the rapid growth of microbial mats over the fish. However, acid etching of concretions containing well preserved fish indicate the mats to have actually been cut and disrupted by the fish (see figs. 2.14, 2.17). Instead, I suggests that the bloated carcasses were prevented from refloating by a time-related change in the sediments consistency triggered by the precipitation of early diagenetic minerals around the decaying carcasses (Berner, 1968a). This is supported by evidence of microfaulting in the laminae directly adjacent to some transgressive fish (see fig. 2.10). The change in consistency ensured that the fish were preserved in three-dimensions. A similar conclusion has been reached in number of other deposits (Martill, 1985; 1993).

The instability of the upper few centimetres of sediment in the Romualdo Lagoon may have been responsible (or partly responsible) for the presence of only a very impoverished benthic fauna. Certainly, soupy substrates have been cited as the cause for the low diversity of the benthic fauna of the Lower Oxford Clay (Duff, 1975).

In contrast, the absence of any evidence of soft sediment disruption in the type 1 concretions (especially transgressive fish) implies this substrate to have been relatively firm.

2) RATE OF SEDIMENTATION

Assuming the soft tissues of fish such as *Notelops* to take several weeks to completely decay (an assumption supported by actualistic experiments with similarly sized fish, see text fig. 8.6), one can calculate a crude estimate for the rate of sedimentation in the Romualdo Lagoon based on the thickness of laminated sediment overlying bedding-plane parallel fish in which gas escape structures occur. This of coarse assumes that sedimentation was not

episodic (this is not necessarily supported by sedimentary evidence, see Section 2.5). Based on five fish from type 2 concretions, this thickness averages 3mm and gives a rate of sedimentation of around 2.6cm/yr. This figure is high (and therefore may well refer to episodic sedimentation) but is not beyond those which have been recorded from comparable modern environments (see Sadler, 1981, fig. 4).

Due to an absence of any relevant taphonomic structures, it has not been possible to estimate the rate of sedimentation in the type 1 concretions.

3) PALAEOSALINITY

Due to the lack of diagnostic fossils (most notably cephalopods, oysters, crinoids, and marine reptiles) there has been little agreement on the palaeosalinity of the Romualdo Member. Both marine (Schaeffer, 1947; Silva Santos and Valenca, 1968; Martill, 1988; Arai and Coimbra, 1990; Baudin *et al.*, 1990) and fresh water (Bate, 1972; Mabessone and Tinoco, 1973; Silva, 1983, 1986a, 1986b) salinities have been invoked. A lagoonal setting in which the salinity of the surface waters switched rapidly from fully marine to brackish is the most widely accepted environmental scenario at present (e.g. Silva Santos and Valenca, 1968; Medeiros, 1990). These fluctuations were probably seasonal and accentuated by pulsatory marine incursions (Arai and Coimbra, 1990). This palaeoenvironmental setting accounts for: a) the impoverished composition of the invertebrate fauna, b) the restriction of marine organisms to specific levels, and c) the apparent interdigitation of marine, brackish, and freshwater organisms at all other levels. I concur with this model and below discuss the salinity of the water just above the sediment surface.

Many of the contradictory conclusions regarding the palaeosalinity of the Romualdo Member can be attributed to the apparent increase in the palaeosalinity of the Santana Formation up sequence (Beurlen, 1964; Berthou *et al.*, 1990; Silva-Telles and Viana, 1990). Overprinting this is the evidence for the sporadic, but considerable influx of freshwater from the Batateiras delta systems into the restricted lagoon. The inflated thickness of the Batateiras Formation in the N and NE of the basin (see text fig. 2.15), and the presence of a

distinctive fauna at one level here - represented by the type 1 concretions - also suggests the palaeosalinity of the Romualdo Lagoon to have varied laterally to some extent.

Furthermore, there is also no reason to assume that the water at the bottom of the Romualdo Lagoon during the growth of the type 2 concretions was of the same salinity as that higher up in the water column. Indeed, there is evidence to support the existence of a halocline (Martill, 1988). In particular:

1) Fish are abundant and taxonomically diverse in the Romualdo Lagoon implying the surface waters to have been highly productive. In contrast, benthic organisms are extremely rare (with the exception of ostracodes) and taxonomically impoverished. Martill (1988, p16) proposed this to be largely the consequence of a hypersaline hypolimnion; sediment consistency (see above) and to a lesser extent oxygen levels (see below) may also have been an important contributory factor.

2) Benthic algal mats are prolific (see Section 2.5). Seilacher *et al.* (1985, p15) have proposed these to be largely restricted to hypersaline environments since high salinities exclude grazing gastropods.

3) Postmortem arching of the vertebral column of *Vinctifer* and *Cladocyclus*, and the excessive fanning of the caudal and pectoral fins of *Notelops* and *Rhacolepis* (see Section 2.5.3) are also indicative of elevated salinities. Taphonomic structures produced in actualistic necrolytic experiments which are identical to those preserved in the soft tissues of fish from the Romualdo Member suggest the salinity of the hyperlimnon to have been approximately twice that of normal marine water (i.e. ≈7%)(see Chapter 6).

A hypersaline hypolimnion is not at all surprising. Floral evidence implies fairly arid conditions to have existed during the deposition of the Romualdo Member (Lima, 1978b), and the basin was located some considerable distance inland (see Section 2.1). Furthermore, at Araripina and Ipubi, little more than 4.5m separates the Romualdo Member from thick evaporites of the Ipubi Formation.

The restricted geographical and stratigraphical position (most notably in the region where the Batateiras deltas reached their greatest development) of type 1 concretions, and the abundance of terrestrial fossils (see Maisey, 1991, p68), implies these concretions to have

developed in an environment different to that of type 2 concretions. The type 1 concretions are dominated by juvenile specimens of *Tharrias*. *Tharrias* is one of only two fish taxa that the Romualdo Member shares with the largely non-marine Crato Formation (pers. comm. P. Brito, 1991, Paris). The abundance of juvenile instars in the taxonomically impoverished ostracode fauna (mostly *Pattersoncypris micropapillosa*) of type 1 concretions, suggests they occupied a transient environment (pers. comm. Mathew Wakefield, British Gas). I therefore propose type 1 concretions to have formed in a near-shore (Maisey, 1991, p70), freshwater or brackish embayment.

4) WATER DEPTH

The abundance of algal mats and stromatolites in the Romualdo Member indicate the sediment's surface to have been at least periodically within the photic zone. Relatively 'shallow' depths (≈ at storm wave base) are also suggested by the frequency with which scours and other minor erosional structures occur in the type 2 concretions. A maximum depth in the order of 30m is probably a realistic estimate; much shallower depths may not have been capable of maintaining the thermo- and/or halocline which was almost certainly present (see above).

Depths probably varied enormously according to the relative position of deltas and topographic features (see Section 2.1). Depths probably also varied with time. Certainly the presence of desication cracks at the base of the Romualdo Member, and bioclastic limestones at its top and bottom imply shallower depths existed during the deposition of these lithologies than for those associated with the type 2 concretions.

5) CURRENT ACTIVITY

There is considerable negative evidence to suggest that for the majority of time, the water just above the sediment surface in the Romualdo Lagoon was stagnant. In none of the fish examined is there the unidirectional sorting of skeletal elements typical of even gentle currents (Elder, 1985, pp45-53). Even the lepidotrichia (the most susceptible elements to disturbance by currents) of all of the fish examined were fully articulated.

These background conditions however, appear to have been intermittently interrupted by periods of relatively intense current activity. These 'event' horizons (presumably storm related) are preserved as scours which concentrated skeletal debris and exhumed the impoverished benthic fauna. Storms were probably also responsible for imbricating gastropods, articulated bivalves, and small fish (fig. 2.30) against other shelly material and even fish (see Section 2.5.3).

6) TEMPERATURE

During the deposition of the Romualdo Member, the Araripe Basin was located near the equator (according to the reconstruction given by Petri, 1987) in a dry climatic belt (Lima, 1978b). This and the enclosed nature of the Romualdo Lagoon implies its surface waters would have been relatively warm. The abundance of disarticulated fish debris in the type 2 concretions further supports this conclusion. Such material is indicative of floating carcasses (Schäfer, 1972, p58) which are typical of temperatures at or above 16^0C (Elder, 1985, p24). This however, because of the postulated presence of a halocline (see above), is no indication of the temperature at the sediment surface where mineralization of the organisms actually took place (see Section 7.2).

Since many fish in the type 2 concretions are fully or partially articulated and appear not to have shifted from their original position of rest (see Section 2.5.3), I assume that many fish instead of floating, sank directly to the sediment surface and subsequently did *not* experience refloatation. At normal marine salinity this would indicate the temperature at the sediment/water interface to be at or below 16^0C (Elder, 1985, p24). However, according to Elder (1985, pp24-36), the buoyancy of dead fish is greatly affected by the ambient salinity; fish carcasses may remain negatively buoyant even above 16^0C in elevated salinities. Martill (1988) has proposed many of the fish to have been ensnared and/or overgrown by algal mats. It is not clear what this, the extremely rapid rates of mineralization (see Section 2.5.1 and Chapter 6), and the depth to which some fish penetrated the sediment (i.e. transgressive fish) would have had on the fishes' buoyancy. Certainly, the bloated morphology of some fish suggests that they did contain sufficient decay gas to have floated freely in the water column. However, the exceptional preservation of these specimens indicates that this did not

occur; they were prevented from doing so and kept at or below the sediment surface. I therefore estimate the temperature at the sediment surface to have been just in excess of 16^0C.

The type 1 concretions developed in a shallow, freshwater or brackish embayment (see above). Most of the fish in these concretions have experienced at least partial floatation (see Section 2.5.3). This suggests the water to have been around 16^0C (Elder, 1985, p24).

7) OXYGEN LEVELS

The abundance of nektonic fish clearly indicates the surface waters of the Romualdo Lagoon to have been well oxygenated. In contrast, with the exception of ostracodes, benthic faunal elements are both taxonomically and numerically sparse. Benthic fish (e.g. *Rhinobatos*) are recorded but are extremely rare, and although fragments of crustaceans are recovered in abundance from concretions containing fish, it is not clear whether these are autochthonous; they may well be coprolitic in origin, or be derived from necktonic or epipelagic decapod shrimps. Evidence of scavenging is extremely sparse, suggesting the diverse fish fauna of the Romualdo Lagoon was unable to venture even temporarily into the hypolimnion.

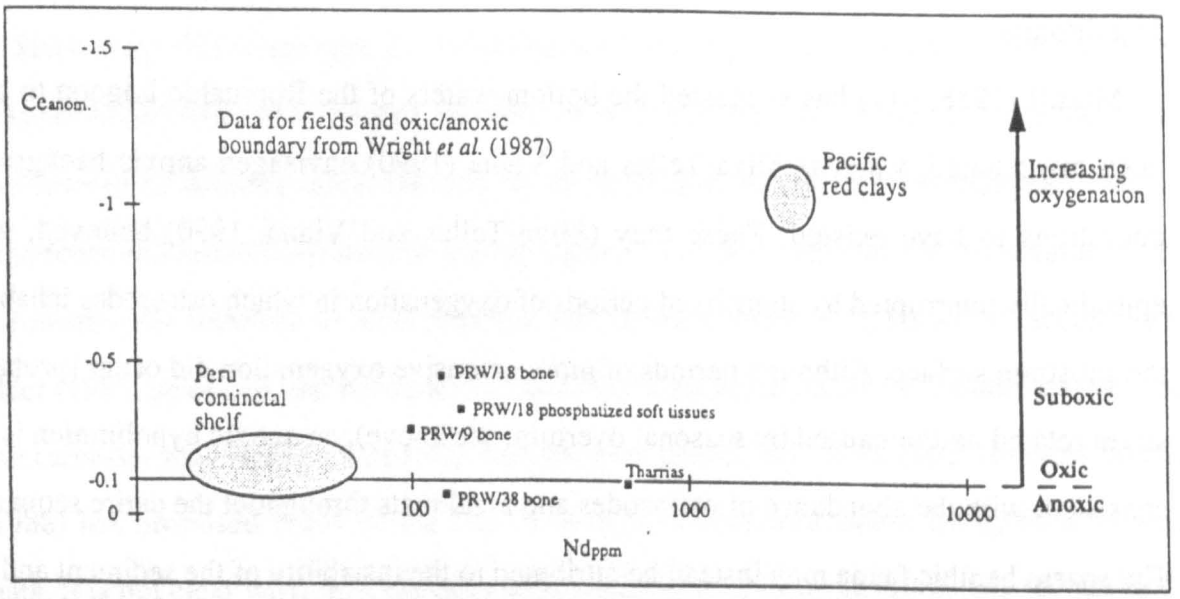
Martill (1988, p11) has suggested the bottom waters of the Romualdo Lagoon to have been oxygenated, whereas Silva-Telles and Viana (1990) envisaged anoxic background conditions to have existed. These they (Silva-Telles and Viana, 1990) believed, were episodically interrupted by short-lived periods of oxygenation in which ostracodes inhabited the substrate surface. Although periods of more extensive oxygenation did occur (probably storm related and/or caused by seasonal overturn, see above), an anoxic hypolimnion is not consistent with the abundance of ostracodes and algal mats throughout the entire sequence. The sparse benthic fauna may instead be attributed to the instability of the sediment and the inhospitable salinity (see above). Indeed, the abundance of scours and other minor erosional features suggests the water column to have been regularly and quite intensely mixed. Such short-lived oxygenation events have been recognised in a number of other laminated, poorly oxygenated (or anoxic) organic-rich sediments (for a review see Savrda *et al.*, 1991).

A dysoxic hypolimnion is most consistent with palaeoenvironmental and rare earth elemental (REE) data. Wright *et al.* (1984) have proposed that under anoxic conditions, all REEs are scavenged from the water column by phosphatic skeletal debris in proportions related to their dissolved abundances. In contrast, under oxic conditions, Cerium (Ce) is oxidized and is preferentially scavenged by hydrous iron oxides thereby creating a distinctive negative Ce anomaly. Such anomalies have been used by Wright *et al.* (1984) as a quantitative measure of the level of oxygenation of various sediments. They define the Ce anomaly as:

$$Ce_{anom.} = \log(3Ce_n/2La_n + Nd_n)$$

where: n=North American Shale (Haskins *et al.*, 1968)-normalized value; La= Lanthanum; Nd= Neodinium.

Analyses (according to the procedure of Wright *et al.*, 1984, p326) of a limited number of bone and phosphatized muscle samples from well preserved fish from type 2 concretions most commonly give a dysaerobic signature (text fig. 2.24). An anoxic signature was given by one sample (PRW/38 bone). This suggests the upper few centimetres of sediment in the Romualdo Lagoon to have been generally dysoxic but to have experienced periods of complete oxygen deficiency.



Text figure 2.24: $Ce_{anom.}$ of skeletal debris and phosphatized soft tissues from the Romualdo Member. PRW/18 are samples from a scour (representing ?oxygenated periods of deposition); PRW/9 and PRW/38 are fully articulated specimens of *Notelops* sp. These are from type 2 concretions. *Tharrias* sp. is from a type 1 concretion.

The REE evidence is supported by the distribution of authigenic pyrite in the type 2 concretions. Many are blue/grey when fresh but weather to a distinctive orange brown. This suggests pyrite to be disseminated throughout the sediment and is indicative of continual steady-state precipitation and therefore anoxic porewaters (Berner, 1980). In many others however, the pyrite coats the bones of fish, is concentrated in gastropods, and/or occurs as framboids in the sediment. Such a distribution is indicative of poorly oxygenated interstitial fluids in which the precipitation of pyrite was restricted to specific reducing microenvironments (Brett and Baird, 1986, fig. 11).

The presence of undisturbed sedimentary laminae and abundant algal mats, and a low diversity benthic fauna consisting of ostracodes, rare bivalves and shrimps, is typical of exaerobic environments (Savrda and Bottjer, 1987). The abundance and diversity of faunal elements in type 2 concretions is however somewhat lower than that predicted by Sageman *et al.* (1991) for such environments. This probably results from the additional environmental stresses imposed on the fauna by the high salinity of the hypolimnion, and the soupy, thixotropic upper sediment. The relative importance of substrate consistency and oxygen levels in controlling the diversity of benthic faunas in black shales is discussed in detail by Wignall and Hallam (1991) and Wignall (1993).

According to Maisey (1991, p70), a somewhat different environment prevailed above the type 1 concretions. He (Maisey, op. cit.) proposed the absence of pollen (which rapidly decomposes in oxygenated environments) and pyrite to be a reflection of the relatively high levels of oxygenation of the substrate. Certainly there is a case for such a statement.

2.5.5 THE CAUSE OF THE FISH MASS MORTALITIES IN THE ROMUALDO LAGOON

Concretions containing more than one fish are abundant in the Romualdo Member. Usually, only a single taxon occurs (usually *Rhacolepis, Vinctifer,* or *Tharrias*) and the size range of the individuals varies very little. This implies these concretions record mass mortality events, rather than the slow accumulation of carcasses over a period of time. The number, extent, and precise cause of these catastrophic events remains to be established.

Indeed, there is no reason to assume the same process to have been responsible for all of the mass mortalities. However, the abundance of fish displaying tetany of the jaws (fig. 2.31) implies death in the majority of cases to have been caused by respiratory stress. Likely causes therefore include anoxia, heat shock, and adverse salinities (Elder, 1985).

Martill (1988, p14) has proposed (amongst other mechanisms, see below) that sudden fluctuations in the temperature of the surface waters were responsible for the mass mortalities. Certainly, mass mortalities resulting from temperature shock have been recorded in modern environments (see Gunter, 1947). However, according to the palaeogeographical reconstruction given by Barron (1987, fig. 6) for NE Brazil, the Araripe Basin lay only a few degrees south of the equator during the Albian, and was therefore unlikely to have experienced wild temperature fluctuations. Furthermore, according to Elder (1985, p34), evidence of tetany caused by heat shock is unlikely to survive the period of floatation which more often that not result from rises in temperature (Elder, 1985, p34).

Later, Martill (1989, fig. 3) proposed the mass mortalities to have been triggered by deoxygenation of the water column. This, he (Martill, op. cit) suggested had been caused by the decomposition of algal blooms whose proliferation had been stimulated by a nutrient input. Such events have been recorded periodically off of the coast of Africa and South America (Brongersma-Sanders, 1948, 1949), but it is difficult to find supportive evidence for such phenomena in the fossil record.

Martill (1988, p14) also proposed that catastrophic changes in the salinity of the surface waters may have been responsible. I endorse this theory. There is considerable evidence in the Romualdo Member for a periodic mixing of the entire water column and therefore a breakdown of the halocline (see above). Indeed, the association of imbricated molluscs with fully articulated fish in some type 2 concretions (see Section 2.5.3) implies a causative relationship between the death of the fish and the mixing of the water column; the exceptional preservation of the fish indicates them to have only recently died at the time of the current activity.

Whatever the cause of mortality, there is some evidence to suggest that the fish arrived at the sediment surface whilst still alive. Along the entire length of one specimen (PRW/4), the body had sunk 5mm into the sediment. In the trunk region, the only evidence of sediment

disturbance is associated with this phenomenon (see fig. 2.17). In contrast, around the head (fig. 2.32) and tail, there is also a lateral element of disturbance which extends under unaffected sedimentary laminae. Since the greatest volume of decay gas in fish is usually produced within the body cavity and not in the head or tail, it might be expected that sediment disruption associated with postmortem shifting of the carcass would be concentrated above the stomach region. This however, is not the case. The pattern of sediment disturbance is more compatible with a distressed and weak fish having thrashed around in the sediment while dying.

The type 1 concretions were almost certainly deposited in a subtly different environment to the type 2 concretions, and the fish were therefore probably exposed to different environmental stresses. However, based on palaeontological and sedimentological evidence (see Sections 2.5, 2.5.2, 2.5.3), I suggest that the *Tharrias* mass mortalities were also caused by salinity stress. These fish probably inhabited short-lived, mixohaline environments close to the Batateiras deltas which were eventually (or periodically) invaded by tongues of more saline water.

2.6 STRATIGRAPHICAL DISTRIBUTION AND ABUNDANCE OF PHOSPHATIZED SOFT TISSUES

Previously, phosphatized soft tissues have been recorded only from the Romualdo Member of the Araripe Basin (see Bate, 1971; Cressey and Patterson, 1973; Martill, 1988). This study has demonstrated phosphatized soft tissues also to occur in the Nova Olinda Member of the Crato Formation (see Section 5.2.3). Their abundance, and lateral and taxonomical distribution however remains to be established.

Regarding the Romualdo Member, although the outcrop of this unit is relatively well constrained (see Ministerio das Minas e Energia D.N.P.M., 1965 1:100000 geological map of the Chapada do Araripe), little is known of the lateral and vertical distribution of phosphatized soft tissues in it (see Chapter 4 for a discussion of the taxonomic distribution). I have recorded phosphatized soft tissues from sites along the majority of the Romualdo Member's outcrop including localities around Ipubi, Araripina, Simões, Barbalha, Jamacaru, Santana do Cariri , Serra da Maozina, Porteiras, and Jardim (see text fig. 2.1).

They are however, most prolific in the NE and E of the basin, particularly around the latter four sites. This, is probably a polarized view of an essentially basin-wide distribution; recorded abundances of phosphatized soft tissues are probably skewed in favour of the E and NE sites due to the prevalence of commercial collecting there.

Constraining the vertical distribution of phosphatized soft tissues is extremely difficult. Access to material on site other than in mine stockpiles and stream debris is limited. However, it is clear that soft tissues are preserved in both types of concretions, but not in the large septarian concretions located towards the top of the Romualdo Member (see Section 2.4.6.3). In addition, phosphatized soft tissues have also been recovered from decapod shrimps preserved in platy limestones interbedded with type 2 concretions in the far west of the basin near Simões. Although a more detailed analysis of the concretions *in situ* is required, it is my opinion that the soft tissues (or at least fish containing a considerable abundance of such material) are restricted to specific concretionary horizons.

In the type 1 concretions, the quantity of soft tissues preserved in individual organisms, and the number of organisms actually containing such material, is rather limited relative to the type 2 concretions. Martill (pers. comm.) suggests ≈20% of type 2 concretions contain appreciable quantities (i.e. visible in hand specimen) of phosphatized soft tissues, whereas I estimate only ≈5% of the type 1 concretions do.

2.7 SUMMARY

The depositional history of the Araripe Basin was controlled by the tectonic heritage of the Brazilian basement and the opening of the southern Atlantic Ocean. Each major sedimentary unit corresponds to a specific phase in the evolution of the rift (table 2.1). The pre-rift phase corresponds to the Cariri Formation, initial rifting or the 'Alagoan Stage' (Brito and Campos, 1982, 1983) to the Missao Velha-, Batateiras- and Crato- Formations (Medeiros, 1990); the Ipubi Formation represents the restricted evaporitic epeiric sea; and the Santana Formation or 'Vinctifer Biozone' (Brito, 1984) corresponds to the marine invasion. The Exu Formation probably corresponds to renewed fault activity. No major unconformaties except for the ones above and below the Cariri Formation are recognised; post-Cariri sedimentation was essentially uninterrupted.

There is a definite increase in the level of marine influence up section (Beurlen, 1964; Berthou *et al.*, 1990; Silva-Telles and Viana, 1990)(see table 2.1). Only minor ingressions occurred in the Missao Velha (i.e. the camadas batateiras, Hashimoto *et al.*, 1987; Baudin *et al.*, 1990; Pons *et al.*, 1990) and Crato Formations (see Section 2.4.4.2), with the first major marine influence occurring in the Ipubi Formation (Berthou *et al.*, 1990; Berthou and Pierre, 1990). The presence of dinoflagellates (Arai and Coimbra, 1990) and echinoderms at the top of the Santana Formation (see Section 2.5.2) indicates near normal marine salinities to have become established prior to the renewed cycle of fluvial sedimentation (the Exu Formation). The correlation of some of the marine ingressions with adjacent basins (see Brito, 1984; Hashimoto *et al.*, 1987) implies at least these to have been under eustatic control.

Formation	Depositional environment	Basin development after Medeiros (1990); Brito Neves (1990)	Marine influence (non-marine / fully marine)	Age (based on data from publications given in the text)
Exu	fluvial	?renewed faulting		Cenomanian
Santana	lagoonal	post-rifting adjustment		?
Ipubi	barred basin			Albian
Crato	hypersaline lagoon	final rifting		Aptian
Batateiras	deltaic	syntectonic		EARLY CRETACEOUS
Missao Velha	fluvio-lacustrine			? LATE JURASSIC
Cariri	fluvial/alluvial	pre-rift		u/c SILURIAN

LATE CRETACEOUS

Table 2.1: The depositional environment and level of marine influence (simplififed) of each major sedimentary unit in the Araripe Basin relative to the evolution of the rift.

The structural, palaeoenvironmental, and ecological characteristics of the Romualdo Member suggest it to have been deposited in a relatively shallow, warm lagoon with a permanent but restricted connection to the Atlantic. Large volumes of fresh water were flushed in (probably spasmodically) by deltas which together with the lagoon's restricted

nature, created salinity fluctuations in the surface waters. These excluded many of the 'typical' Cretaceous marine organisms found elsewhere.

A persistent halocline separated the dysoxic, hypersaline bottom waters of the Romualdo Lagoon from its well oxygenated, warm surface waters. For much of the time, the soupy substrate was sparsely populated by salinity tolerant ostracodes, shrimps, bivalves, and gastropods, and the hypolimnion was stagnant. These background conditions were interrupted by storms which mixed the hypolimnion and epilimnion, and induced repeated fish mass mortality events.

In the shallows at the margins of the lagoon, bioclastic limestones and stromatolites developed. In certain areas, a restricted fauna consisting of ostracodes and *Tharrias* (represented by the type 1 concretions) lived until overcome by tongues of water of different salinity.

2.8 CONCLUSIONS

1) Sedimentation in the Araripe Basin was closely linked to rifting events associated with the opening of the northern part of the South Atlantic ocean. The basin experienced episodic marine invasions which increased in frequency and size towards the top of the succession.

2) Phosphatized soft tissues occur both in the Crato Formation and Romualdo Member. In the Crato Formation, according to the limited data available, soft tissues appear to be restricted to the Nova Olinda Member. In the Romualdo Member, phosphatized soft tissues are extremely abundant and occur across the entire extent of its outcrop. They are not restricted to a specific concretionary horizon but are more common in type 2 concretions than type 1 concretions.

3) The Crato limestones were deposited in an inhospitable lagoon with anoxic bottom waters. The lagoon had a tentative and probably temperamental marine connection, and became increasingly more saline up sequence. The salinity of the surface waters fluctuated greatly from hypersaline to nearly fresh according to the extent of the marine connection and the volume of fresh water seasonally flushed in by the Batateiras deltas.

4) The shales of the Romualdo Member were deposited under a halocline. Background conditions at the sediment surface were dysoxic. Periodic disruptions of the halocline were probably responsible for the mass mortalities of the diverse ichthyofauna.

5) Sedimentation was episodic in the Romualdo Lagoon, and over short time intervals may have been as high as 2.6cm/yr. The presence of transgressive fish indicates the sediment to have been soupy. Acid digestions suggest sediment porosities to have been as high as 92% at the sediment surface.

6) Each fish taxon exhibits a distinctive taphonomy. Many fish in the type 2 concretions did not experience refloatation but are excessively bloated by decay gases. They were therefore probably restrained by the sediment, algal mats, and/or very early mineralization. This suggests the temperature at the sediment surface to have been just over 16^0C.

7) The fish were calcified extremely rapidly after their incorporation in to the sediment. Some concretions (or at least possible adipocere precursors) and the first calcite generation within the fish, developed within a matter of weeks of the fishes' death.

CHAPTER 3
MINERALOGY AND MICROFABRICS OF SOFT TISSUES FROM THE ROMUALDO MEMBER BIOTA

3.1 INTRODUCTION

In order to be able to establish the processes responsible for the phosphatization of soft tissues in the Romualdo Member, a detailed knowledge of the microfabrics replacing the soft tissues is essential. Only with such an understanding can one comment on the relative importance of mechanisms of microbial- and inorganic-mineralization, and on the level to which the organic substrates controlled crystal nucleation.

Phosphatized soft tissues have been demonstrated to be replaced by a number of different microfabrics (e.g. see Pinna, 1985; Müller and Walossek, 1987; Willems and Wuttke, 1987; Allison, 1988d; Micklich and Wuttke, 1988; Schultze, 1989) but there has been little agreement as to each microfabric's genesis. This is in part due to the lack of a single, all encompassing investigation of their petrography at magnifications capable of elucidating their provenance. This chapter describes the microfabrics replacing the soft tissues of the Romualdo Member. Interpretations of their genesis are based largely on comparisons with structures that have been produced *in vitro*, and that have been recorded from phosphorites.

Martill (1990b) suggested that phosphatized soft tissues represent the most exceptional material so far available to palaeontologists. The justification for this statement is tested by comparing the ultimate resolution of detail that each fabric is capable of preserving with that characteristic of other mechanisms of soft tissue preservation.

3.2 MINERALOGY OF SOFT TISSUES FROM THE ROMUALDO MEMBER

The habits of crystal microfabrics are dictated by their crystallography and therefore their composition. Thus, once the the mineralogy of the crystallites and crystal-aggregates replacing the soft tissues of the Romualdo Member biota is identified, inorganic precipitates

may be distinguished from lithified microbes, and the importance of organic substrates in nucleating the crystallites may be determined.

Bate (1972), Cressey and Patterson (1973), and Martill (1988, 1990b, text fig. 1) have all identified the mineral phase replacing the soft tissues of fossils from the Romualdo Member as apatite. Schultze (1989), however, has rejected Martill's (1988) results despite having accepted that some of the ostracodes (analysed by Bate, 1972) and all of the copepods thus far described (Cressey and Patterson, 1973) are replaced by a phosphate. Schultze (1989) believes the soft tissues of fish from the Romualdo Member to be replaced by calcite. This led Maisey (1991) also to question Martill's (1988) original conclusions.

Schultze's (1989) contention must be treated with some scepticism. He mechanically prepared his material for analysis (Schultze, pers. comm. 1992) and thus is likely to have swamped the apatite signatures of the phosphatized soft tissues with those of the volumetrically more important diagenetic calcites which surround the soft tissues and form the body of the concretions (see Section 2.5.1). I have never observed calcite to pseudomorph the soft tissues of the Romualdo Member biota; calcite occurs only as a cavity-fill cement. My XRD[1] analyses of fossilized muscle (text fig. 3.1), dermis, gills, coprolites and portions of the alimentary tract from several specimens of the fish - *Notelops* and *Rhacolepis* - confirm an apatitc composition. The peaks closely match those of standard samples of calcium hydroxyapatite (HAP) with the composition ($Ca_5[PO_4]_3[OH]$). The tissues analysed were recovered from acid (10% Acetic) digestions after more than a weeks exposure. An apatitic composition is also supported by EDAX elemental analyses[2] of similarly treated fossil arthropod material (Wilby and Martill, 1992, pp31-32).

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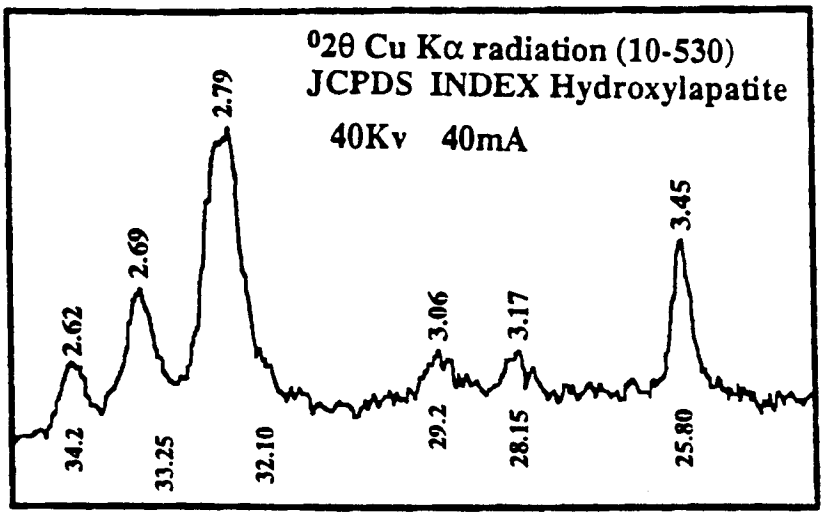
[1] XRD is a standard and widely utilized technique of identifying crystalline phases, although poorly crystalline minerals and mixtures of minerals can be difficult to identify. SEM examination of soft tissues from the Romualdo biota indicate that only a single crystalline phase is present.

[2] Note: EDAX elemental analysis give only those elements present within the mineral phase and do not give either their abundance or crystallographic relationship. However, the presence of only calcium and phosphorus in samples suspected to be apatitic can provide valuable supporting evidence.



Text figure 3.1: XRD pattern of muscle from a *Notelops* sp. (PRW/9). The peaks are characteristic of hydroxyapatite.

3.3 CRYSTAL MICROFABRICS OF SOFT TISSUES FROM THE ROMUALDO MEMBER

Martill (1988, 1990a, 1991) has identified several microfabrics replacing soft tissues from the Romualdo Member biota. Although illustrating that a number of processes are responsible for their mineralization (see below), this belittles the true degree of variation. Presented below is a description of the variety, structure and genesis of crystal microfabrics that I have observed replacing the soft tissues of the Romualdo Member biota.

Two distinct categories can be recognised; one in which the tissues are replaced by phosphatized microbes, and other in which the tissue's original ultrastructure is replaced by individual crystals and/or crystal aggregates which are not directly referable to microbes. Hirschler *et al.* (1990a; 1990b, p47) have proposed that both of these microfabrics have a microbial origin. They demonstrated experimentally that when microbes are supplied with an organic source of phosphorus (e.g. RNA) and a crystalline or dissolved source of Ca^{2+}, substrates may be replaced either by phosphatized microbes or by crystal aggregates identical to those precipitated *in vitro* from supersaturated solutions (Eanes and Meyer, 1977; Nancollas, 1982). Hirschler *et al.* (1990b) termed the process by which the microbes themselves are mineralized - "biologically controlled" mineralization - following the terminology of Lowenstam (1981) for the mineralization of soft tissues under strict cellular control. Although this is a correct definition of the process by which the microbes

themselves are mineralized, it does not adequately describe the process by which the soft tissues of the decaying host organisms are fossilized. I therefore abandon this term in favour of 'microbial mineralization' (or phosphatization). I define microbial mineralization as: the preservation of soft tissues in a decomposing organism as a direct result of the precipitation of apatite in and/or on microbes infesting those tissues. That is, the soft tissues of the decaying macro-organism are themselves not mineralized, but rather the infesting microbes are.

Hirschler *et al.* (1990b) termed the process by which substrates are replaced by crystallites precipitated external to the infesting microbes as "biologically induced" mineralization, following the terminology adopted by Lowenstam (1981) to describe the incidental precipitation of minerals resulting from the activity of living organisms (e.g. respiration, release of metabolic wastes etc). Certainly, microbes are essential to the release of phosphorus from organics (see Lucas and Prévôt, 1984; Benmore *et al.*, 1983; El Faleh, 1988; Prévôt *et al.*, 1989; Hirschler *et al.*, 1990a; 1990b) and therefore have a role in the phosphatization of all soft tissues. However, contrary to Hirschler *et al.* (1990b), it is not possible to prove that microbes were intimately involved in the phosphatization of those soft tissues from the Romualdo Member which are replaced by crystallites and crystal aggregates which closely resemble inorganic precipitates (see Eanes, 1980). Indeed, actualistic taphonomic evidence (see Section 6.2.1.2) and the lack of any associated signs of microbial activity (e.g. mineralized microbes or 'scars' left by microbes metabolising the tissues, see Section 3.3.1) suggest that microbes, contrary to the experiments described by Hirschler *et al.* (1990b), were not in the immediate vicinity of these tissues. Furthermore, there is some evidence to suggest that rather than having been *induced* by microbes, mineralization of the host organism was *controlled* by the tissues of that organism (see Section 8.3.1). Hirschler *et al's.* (1990b) term for this style of mineralization has therefore the potential for confusion. For these reasons, and the fact that "biologically induced mineralization" *sensu* Lowenstam (1981) refers to mineralization in living systems, I reject Hirschler *et al's.* (1990b) terminology in favour of the term - 'inorganic mineralization'. I define inorganically mineralized (phosphatized) soft tissues as: those tissues in a decomposing organism that

have been replaced by microfabrics which can be precipitated *in vitro* from supersaturated solutions by 'simple' inorganic precipitation (see Eanes, 1980).

3.3.1 DISTINGUISHING BETWEEN MICROBIAL AND INORGANIC MICROFABRICS

Examples of fossilized microbes associated with exceptionally well preserved macro-organisms have been widely reported (e.g. see Wuttke, 1983; and Martill, 1987). The identification of phosphatized microbes however, is not as straight forward due to their superficial resemblance to certain inorganically precipitated crystal aggregates (see Allison, 1988d; Hirschler, 1990b). Despite this, a number of cases of soft tissues pseudomorphed by phosphatized microbes have been recorded (e.g. see Willems and Wuttke, 1987; Micklich and Wuttke, 1988; Clark, 1989; Mehl, 1990; Schmitz, 1991; Martill and Wilby, 1993a). The characteristic features of these, together with microbes recorded from phosphorites (see O'Brien *et al.*, 1981; Riggs, 1982; Soudry, 1987, 1992; Soudry and Lewy, 1988), form the basis of a 'check-list' for identifying micro-organisms in soft tissues from the Romualdo Member.

A number of features are particularly indicative of phosphatized micro-organisms. These include:

1) Phosphatized microbes usually infest only the peripheral tissues of carcasses where access to the external environment is unimpeded (e.g. gills and skin), or are associated with typically bacteria-rich tissues and organic material (e.g. the alimentary tract and faeces).

2) They typically have either: a) an extremely smooth external surface suggesting mineral deposition occurred within a membrane-bound space, or, b) a rather complex, regular arrangement of external projections surrounding a smooth-walled hollow sphere, implying an external coating. Both varieties frequently consist of equidimensional and preferentially aligned crystallites, implying apatite nucleated directly onto a specific organic template.

3) The presence of a number of smaller internal spherical bodies within the microbe which delineate the position of internal membranes and subcellular bodies.

4) A resemblance to communal growth patterns typical of modern bacteria, including dividing pairs, chains, and, coalesced mammillated forms.

5) Their incapability of pseudomorphing the fine ultrastructure of the substrate (see Section 3.4). This reflects their comparatively large size relative to most subcellular structures of metazoans, their association with degraded material, and their prevalence as external coatings.

6) Their association with 'pits' and 'scars' (which mimic the external morphology of the microbes) suggesting active metabolism of the host substrate (e.g. Clark, 1989).

Microfabrics which cannot be defined by the criteria outlined above, I considered to be inorganic in origin (see Section 3.3.4). Inorganic microfabrics similarly have a number of very distinctive features which permit their non-microbial genesis to be substantiated. These are:

1) Inorganic microfabrics are identical to crystal microfabrics precipitated *in vitro* from solutions supersaturated with respect to apatite (see Eanes, 1980).

2) They preserve soft tissues with exceptional precision (see Section 3.4). Even macromolecular details may be preserved (Martill, 1990a).

3) Replacements are considerably more common than coatings (see text fig. 5.6).

4) The position of most inorganic precipitates is structurally dictated (see text fig. 3.3). Any preferential alignment of crystallites corresponds to that of biomolecules in the fossilized tissue (see Section 4.2.2.1).

3.3.2 LITHIFIED MICRO-ORGANISMS IN THE SOFT TISSUES OF THE ROMUALDO MEMBER BIOTA

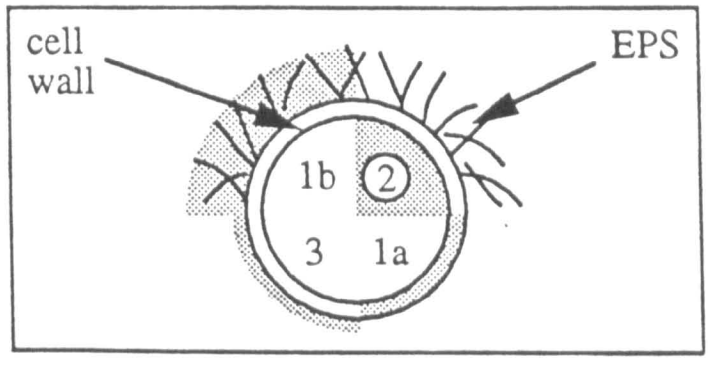
Martill (1988, see Plate 3, fig. 4; and 1991) and Martill and Wilby (1993a) have already described phosphatized microbes from the Romualdo Member. These display three broad styles of mineralization which are not necessarily mutually exclusive (see text fig. 3.2 for summary).

1) Membrane replacement: This involves the selective replacement of the microbe's membrane(s) in preference to any other substrates. This results in a hollow sphere or ellipsoid reflecting the microbe's original dimensions (text fig. 3.2: 1a). Usually, the thickness of the mineralized zone is significantly greater than that of a simple membrane,

suggesting phosphatization to have taken place largely within the surrounding extra-polymeric-substance (EPS) (text fig. 3.2: 1b).

2) Internal mould: The entire microbe is replaced, producing a solid body with dimensions reflecting the cell's original size (text fig. 3.2: 2).

3) Surface coating: Precipitation of apatite does not occur *in* any of the soft tissues of the microbes, but rather occurs as an even 'blanket' on its surface (external to the cell and EPS)(text fig. 3.2: 3). This results in the exaggeration of the microbe's original dimensions to an extent dependent on the thickness of the coating. Frequently, adjacent microbes are blanketed by an unbroken layer of crystallites.



Text figure 3.2: Common styles of bacterium mineralization (shading indicates mineralization). 1a) Membrane replacement; 1b) Mineralization of EPS; 2) Internal mould; 3) External coating. See text for details.

Identification of microbial species has not been possible. The micro-organisms are therefore referred to as 'morphotypes' (refer to table 3.1 for a summary of the structure of each morphotype):

COCCOID MICROBES:

MORPHOTYPE 1: Phosphatized EPS.

Two distinct but structurally very similar microbes with mineralized EPS have been identified in the soft tissues of the Romualdo Member biota. These are discussed separately below:

a) This form is by far the most frequently encountered microbe in the soft tissues of the Romualdo Member, and has been briefly described by Martill and Wilby (1993a). It is composed of hollow spheres, 2 to 3µm in diameter, which frequently impinge on one

another to produce a smooth, mammilliform external surface (fig. 3.1). The hollows are 1 to 1.5μm in diameter with smooth walls. The surrounding mineralized 'envelopes' are usually ≈1μm thick and consist of an external (≈0.3μm thick) layer of acicular crystallites (≈130nm long) arranged at a slight tangent to a thicker basal layer composed of more equidimensional crystallites (≈20nm max. dimension) (fig. 3.2). Circular apertures (averaging 1μm in diameter) which are internally skirted, and bowl-shaped depressions are common (see figs. 3.1 and 3.2). These connect the interior cavity to the external environment. The thickness of the mineralized envelope, and their smooth external and internal surfaces suggest them to be phosphatized EPS (or mucilagenous zone).

Occasionally, this morphotype forms 'strings' and protuberances which bridge voids (fig. 3.1 arrowed). These are not original structural features of the substrate tissue, but rather manifestations of decay. Adjacent microbes within the strings and mammillate forms are either partitioned by a mineralized wall, or are all internally connected. Similar strings of microbes have been figured infesting ostracodes from the same deposit (Bate, 1972, Plate 67, fig. 1).

This morphotype is frequently associated with a series of extremely thin (≈250nm), straight, cylindrical depressions which are rarely greater than 3μm in length (fig. 3.3). These incise several capsules of the coccoid microbe and occasionally occur as simple hollow tubes rather than as incisions. Although they are always associated with morphotype 1a microbes, their rather irregular distribution is not consistent with them being outgrowths (e.g. pili or flagella) of the coccoid microbes. Despite a crude resemblance to certain microborings (e.g. Martill, 1989c), their occasional occurrence as body fossils dismisses such an affinity. Instead, they probably represent the external moulds of an as yet unidentified coexisting micro-organism.

b) Much like morphotype 1a, this microbe consists of hollow spheres 1.5 to 3μm in diameter with circular apertures (1.5μm diameter) and abundant spherical pits and depressions (300nm to 1μm in diameter). It is distinguished from morphotype 1a by the possession of a distinctive 'cauliflower-like' external surface suggesting mineralization

occurred within well defined 'segments' of the EPS (fig. 3.4). This microbe frequently forms sinuous 'strings' between vertical constraints such as adjacent fish scales (fig. 3.5).

Similar globular structures with apertures, assigned to a microbial origin, have been figured from oceanic polymetallic concretions (Janin and Bignot, 1983, Plate 1, figs. 6, and 9-12) and from phosphorites of the Late Cretaceous Mishash Formation, Israel (Soudry and Lewy 1988, Plate 2, fig. c). However, the internal structure, the thickness of the coating, and the external morphologies of these microbes are not identical to those of morphotypes 1a and 1b. Gross morphological similarities therefore probably reflect comparable mechanisms of mineralization rather than genetic relationships.

MORPHOTYPE 2: Internal moulds.

This morphotype consists of perfectly spherical bodies (1μm in diameter) with an extremely smooth external surface. They may either be entirely isolated from one another, *just* impinge on one another, or form dense mammillate 'mats'.

Under SEM they superficially resemble inorganically precipitated microspheres (see Section 3.3.4.2), but may be distinguished on the basis of their occurrence only as coatings and/or very crude replacements of the host tissues (unlike inorganic precipitates). In TEM they are morphologically distinct from inorganic microfabrics. Crystallites of 30nm maximum dimension are arranged radially normal to the microbe's membrane and are comparatively densely packed (fig. 3.6). Some have a very thin (30nm), partially mineralized outer layer separated from the main sphere by a 50nm thick non-mineralized gap (the microbe's membrane). These features, together with the absence of internal cavities suggests this morphotype to be the internal moulds of a micro-organism.

The enormous variations in gross morphology displayed by this morphotype is dictated largely by the size of the microbial population and the topography of the underlying substrate. Where numbers of individuals are low, they are unlikely to impinge on one another. Where colonies are more established, individuals are more likely to experience mutual compression and develop into polygonal globular bodies (fig. 3.7). These resemble the coccoid cyanophytes described by Hofmann (1976, Plates 5 and 6) and Golubic and Hofmann (1976, Plate 2) from Precambrian and Holocene stromatolites, and the micro-

organisms figured by Soudry and Lewy (1988, Plate 1, figs. d and e; and Plate 3, fig. d) from the Lower Cretaceous Mishash Formation of Southern Israel. Some bacteria which have been phosphatized *in vitro* also closely resemble morphotype 2 microbes (Hirschler *et al.*, 1990a, fig. 2; and, Lucas and Prévôt, 1984).

MORPHOTYPE 3: External coatings.

This morphotype consists of spherical, hollow bodies, most commonly 20μm in diameter which have an extremely uneven, granular external coating never more than 500nm thick (fig. 3.8). These probably represent colonies of microbes consisting of many individuals. The coating forms a continuous 'blanket' over the colonies so that microbes within invidual colonies are interconnected. A limited number of the colonies contain one or two tabular crystals (?barytes) which may reach 20μm in maximum dimension.

This morphotype has only been identified from the soft tissues of one fish (PRW/20) in which it was relatively abundant. It has not been identified in TEM.

MORPHOTYPE 4: Internal moulds and phosphatization of the EPS.

Individuals of this morphotype vary between 1 and 2μm in diameter and have a distinctive framboidal morphology caused by the development of large numbers of protrusions from their external surfaces (fig. 3.9). Each protrusion is composed of a mass of densely packed, 50nm long, radiating crystallites. These form a coating around a smooth-walled spherical interior which may be up to 1μm in diameter (fig. 3.10). Internally, some of the microbes contain a mineralized sphere (usually located off-centre) which itself frequently contains another concentrically located sphere inside. Each of these spheres is separated from the others by a less heavily mineralized zone (fig. 3.11). Usually, each microbe is separated from its neighbour by a thin gap. Colonies of this morphotype therefore never form continuous hollow connected structures.

The thickness and ordered arrangement of crystallites in the irregular outer layer of this morphotype, suggests the EPS to have been mineralized. The internally located phosphatized spheres are almost certainly internal and external coatings of a single membrane-bound subcellular body.

Identical structures to which a microbial origin has been given are figured from coprolites from the Eocene oil shales of Messel, Germany (Schmitz, 1991; Plate 20, figs. c, d and, e) and from Late Cretaceous bedded phosphorites in Israel (Soudry, 1992, fig. 4b-f). They have also been described by Riggs (1982) from Neogene phosphorites on the American continental shelf.

MORPHOTYPE 5: Internal moulds and external coating.

This morphotype consists of densely mineralized spheres, up to 4μm in diameter which form mammillate, polyhedral, globular masses. Individual spheres are perforated by apertures identical to those described for morphotype 1a. Each 'globule', which may exceed $8000\mu m^3$, consist of many individuals all of which are internally connected. These are preserved both as solid internal, and thin (500nm thick) external moulds. The space between (probably representing the site of the membrane) is a constant 1μm thickness (fig. 3.12). Morphotype 5 has not been identified in TEM and therefore the size and arrangement of crystallites replacing this microbe are not known.

This morphology is suggestive of competitive growth between individuals resulting from space limitations. In extreme cases, the globules approach the densely packed polyhedral morphologies reported by Lucas and Prévôt (1984) from *in vitro* mineralized microbes, and the fossil micro-organisms figured by Soudry and Lewy (1988, Plate 1, fig. e) from the Late Cretaceous Mishash Formation of Israel.

A similar morphotype composed of both a thin external coating (extremely smooth) and a solid internal cast, is figured extensively by Cressey and Boxshall (1989) in copepods from the Romualdo Member. It however, unlike the strict definition of morphotype 5 (see above), occurs not only as 'polyhedrally-packed' masses but also as isolated individuals (Cressey and Boxshall, 1989, figs. 19, 33 and 55). Typically, this form has an extremely low relief mammillate morphology (Cressey and Boxshall, 1989, figs. 42 and 46), particularly when on substrates with an especially smooth surface (e.g. arthropod carapaces or fish scales). It is therefore frequently difficult to distinguish from the original substrate.

ROD-SHAPED MICROBES:

MORPHOTYPE 6: Internal moulds.

This morphotype consists of ovoid individuals of constant size (1.5μm long, 1μm deep) with a smooth external surface. Usually, each microbe remains directly in contact with only one or two of its immediate neighbours. In some cases, contact is along their long axes and it appears that they were mineralized during cell division; whilst in others they abut against one another to form strings of individuals all with their long axis parallel to certain structural features of the substrate tissue (fig. 3.13).

Similar structures interpreted as bacteria have been recorded extensively in the palaeontological and biochemical literature (see for example Willems and Wuttke, 1987, Plate 5, figs. 3 and 4; Micklich and Wuttke, 1988, fig. 8; Clark, 1989, fig. 1c and d; Hirschler *et al.*, 1990a, figs. 5a, b and c), and the peculiar disposition of bacteria to align themselves parallel to one another has also been documented (O'Brien *et al.*, 1981). Morphotype 5 morphologies are therefore considered to represent the internal moulds of rod-shaped bacteria.

3.3.2.1 NOTE ON THE IDENTIFICATION OF RECENT BACTERIAL CONTAMINANTS IN FOSSIL SOFT TISSUES

Contamination of fossil soft tissues by Recent microbes is a serious problem, particularly in material which has been stored for long periods (see Allison, 1990a for a review). Non-mineralized Recent microbes may be distinguished from mineralized examples under SEM by their rapid distortion/rupture when exposed to accelerating voltages in excess of 15Kv, and by their excessively smooth ('clean') external surfaces. In cases where such contamination is suspected to be extensive, it may also be possible to distinguish Recent microbes from fossil ones with C^{14} analysis (using laser mass spectrometry). Mineralized Recent microbes however, may only be distinguished from fossil ones by careful examination of their relationship with the tissue. Recent micro-organisms are unlikely to form coatings which are separated from the substrate (a common occurrence in fossil material, see fig. 5.19), or to 'bridge' wide (>5μm) gaps unsupported (as do fossil microbes see fig. 3.1), since in both cases an organic substrate is required. Recent microbes are more

likely to disrupt and fracture the friable fossilized soft tissues through their settlement and subsequent growth within confined interstices.

Recent microbial contaminants may therefore be quite easily and confidently distinguished from fossil examples.

3.3.3 MECHANISMS OF MICROBIAL PHOSPHATIZATION

Despite having received considerable attention, the mechanisms responsible for the mineralization of microbes are not well constrained. Variations in the size and arrangement of crystallites between microbes (see Section 3.3.2) suggests mineralization to be dictated by the specific properties and composition of their mucilaginous capsules (Soudry and Champetier, 1983, p420; Lewin 1990, p539). The processes involved in the deposition of crystallites external to the plasmalemma are likely to be wholly different to those responsible for the precipitation of minerals internally.

Ennever *et al.* (1976) suggested that the capability of bacteria to mineralize correlates directly with the presence or absence of specific membrane-bound proteolipids which act as nucleation sites. Although the importance of proteolipids as sites of apatite nucleation is maintained by most authors, their universal occurrence as membrane components even in microbes which *apparently* do not mineralize, led Ennever *et al.* (1981, 1986) later to question their singular relevance. Instead, they (Ennever *et al.*, 1981, 1986) proposed cytoplasmic electrolyte levels and in particular Ca^{2+} and Mg^{2+} to be of more fundamental importance, phosphatization only being initiated when transmembrane ion pumps become inactive (due to dormancy or the death of the cells) and Ca^{2+} and dissolved phosphorus are free to leak back in to the cell. The effect of Mg^{2+} on the precipitation of apatite is discussed in Section 3.3.5 and has been experimentally investigated in microbial systems by Prévôt *et al.* (1989).

In contrast, other models positively depend on the metabolism of the cells. Lewin (1990) suggested external moulds of Chlorophyta to be produced by biologically induced pH changes, leading to a shift in the stability of dissolved Ca^{2+} and PO_4^{3-} ions.

Hirschler *et al.* (1990a) have also suggested that the role of the bacterium membrane may be over stated. They demonstrated experimentally that apatite may be synthesized from

nucleotides using bacterial alkaline phosphatases in the absence of microbial membranes. This suggests that the action of microbial enzymes in releasing organically-bound phosphates is more important to the process of phosphatization than is the presence of the microbe as a nucleation site. Indeed, Lucas and Prévôt (1992, p399) have intimated that almost all enzyme-producing micro-organisms are capable of mediating apatite crystallization; the determining factor is whether their enzymes are released during life or during autolysis.

3.3.4 INORGANIC MICROFABRICS REPLACING THE SOFT TISSUES OF THE ROMUALDO MEMBER BIOTA

Except in a few tissues where microbes may be exceedingly abundant (e.g. the gills and dermis of fish, see Section 4.2.2.1), microfabrics indicative of inorganic mineralization are far more abundant in the soft tissues of the Romualdo Member biota than are mineralized microbes. Inorganic microfabrics may be divided in to three broad groups. These are: 1) gas vesicles; 2) inorganic precipitates in which the crystallites are aggregated in to more or less spherical bodies or microspheres (*sensu* Pautard, 1981); and, 3) inorganic precipitates in which the crystallites are essentially independent entities:

3.3.4.1 GAS VESICLES

Structures interpreted as gas vesicles have only been encountered in coprolites. The distribution of these spherical, hollow bodies is extremely chaotic, and mineralization is restricted entirely to the external surface (fig. 3.14). Unlike fossilized microbes (see Section 3.3.2), the diameters of the bubbles vary enormously from a few hundred nanometres to several microns. The thickness of the mineralized coating is similarly variable, and depends largely on the proximity of adjacent bubbles.

Similar structures also interpreted as mineralized gas vesicles have been recorded in suspension in Lake Kivu (Degens *et al.*, 1972), from various vadose environments (Meyers, 1987; Soudry and Southgate, 1989), and in oolitic ironstones (Dahanayake and Krumbein, 1986).

3.3.4.2 INORGANIC MICROSPHERES

These spherical or subspherical crystal aggregates averaging 800nm in diameter were first identified replacing soft tissues from the Romualdo Member by Martill (1988). Although under SEM they appear largely homologous, when examined in cross-section and at high magnifications under TEM, several distinct morphologies may be identified (refer to table 3.1 for a summary of the characteristics of each type of microsphere). These are:

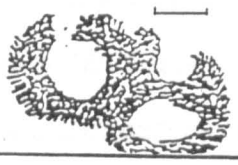
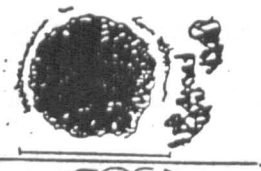
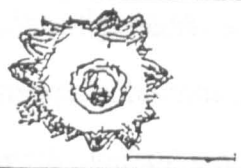
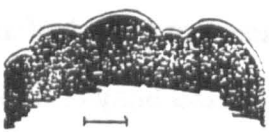
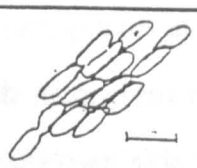
1) Spherical to ovoid bodies averaging 600nm in diameter which frequently impinge on one another and have a hollow or lightly mineralized centre. Although the outer surface may be relatively smooth, the 'wall' of the hollow centre, unlike all mineralized microbes, may be fairly irregular. The crystallites are arranged in a dense radiating fashion normal to the cavity (fig. 3.15) and are of a relatively constant size within individual spheres. Between different fossil specimens however, the crystallites may vary from 30nm to 400nm in length.

2) 1μm diameter, subspherical solid aggregates composed of randomly orientated acicular crystallites 100nm in length (fig. 3.16). No central body or difference in the density of mineralization is obvious.

3) Near perfectly spherical solid masses 600nm to 1μm in diameter, composed of densely packed radiating crystallites of constant size (≈100nm) (fig. 3.17). These microspheres frequently impinge on one another to form aggregated dense masses, although individuals may still be identified.

4) Rather irregular spherical masses each up to 800nm diameter which often coalesce to create larger bodies. These are composed of a fine grained core (crystallites<20nm), approximately 300nm in diameter, from which acicular, 200nm long crystallites protrude (fig. 3.18).

Examples of almost all soft tissues have been identified as having been replaced, or more rarely coated by microspheres. This may either be by dense coalesced aggregates, or by a more open fabric (Martill *et al.*, 1992). Although in SEM some may superficially resemble microbes, microspheres are generally smaller, frequently have a less regular arrangement of crystallites, and they more precisely replicate the tissue's original ultrastructure than microbes.

	Microbe Morphotype	Ultrastructure
COCCOID MICROBES	1 Phosphatized EPS	
	2 Internal moulds	
	3 External coatings	
	4 Internal moulds & phosphatized EPS	
	5 Internal moulds & external coatings	
ROD-SHAPED MICROBES	6 Internal moulds	

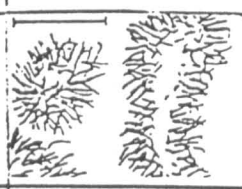
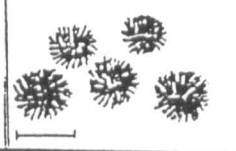
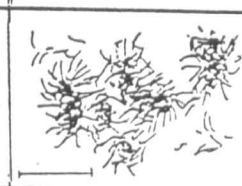
Microsphere morphologies	Ultrastructure
1	
2	
3	
4	

Table 3.1: The ultrastructure of the various microbes and inorganic microspheres replacing the soft tissues of the Romualdo Member biota. Scale bars = 1μm.

3.3.4.3 INORGANIC NON-SPHERULITIC (MICROGRANULAR) MICROFABRICS

Soft tissues replaced by non-spherulitic microcrystalline apatite were first reported from the Romualdo Member by Martill (1990a). TEM examination of such material in the present study has demonstrated that the individual crystallites may be extremely small; often less than 20nm. Usually these are randomly orientated and of a constant size (fig. 3.19). However, structurally orientated crystallites (see Section 4.2.2.1), and fabrics intermediate between inorganic-microspherulitic and inorganic-microgranular may occur. The latter are characterized by irregularly shaped crystal aggregates composed of extremely finely crystalline (<20nm) masses up to 500nm in diameter, surrounded by larger (100nm long) crystals irregularly spaced normal to their surface.

In the Romualdo Member, soft tissues replaced by microgranular apatite preserve the greatest resolutions (see Section 3.4) and are more abundant than those replaced by inorganic microspheres or microbes. Non-spherulitic coatings are also common and frequently preserve the organelles of soft tissues in exceptional detail (see Section 4.2.2.1). An understanding of the processes which favour the precipitation of granular apatite over inorganic microspheres is therefore of paramount importance.

3.3.5 THE PRECIPITATION OF INORGANIC MICROFABRICS

Microspheres: Martill (1988) considered microspheres replacing the phosphatized soft tissues of the Romualdo Member to have precipitated a few seconds after bacteria had metabolized the tissue's phosphorus-rich organics and produced a short-lived microenvironment supersaturated with the released phosphorus. Mineralization was thus intimately associated with the progressive advance of bacterial colonies along the surface of the tissues. Later, Martill (1991) refined this model and suggested that the microspheres may actually represent the waste products of bacterial metabolism which were extruded as *discrete packets.*

Hirschler *et al.* (1990a, 1990b), Azam and Cho (1987) and Martill (1988) similarly proposed microbes to be intimately involved in the precipitation of microspheres. Hirschler *et al.* (1990a, 1990b) demonstrated experimentally that microspheres of similar appearance (although not identical) to those replacing the soft tissues of organisms from the Romualdo Member may develop external to microbes by the local concentration of phosphorus released from an organic substrate by alkaline phosphatases.

Allison (1988d) regarding the phosphatized soft tissues of teuthids from the Oxford Clay Formation, similarly considered inorganic precipitation to be a possible mechanism of producing microspheres. In his model (Allison, 1988d), proteolipids released to the pore waters from decaying cell membranes acted as nucleation sites. In tissues where proteolipids were abundant, nucleation sites would be concentrated and a large number of crystals of similar size would form to produce a microsphere.

Specific sites of nucleation on organic substrates were also considered by Schultze (1989, p193) to have been important in the phosphatization of soft tissues in fish from the

Jurassic of Chile. However, despite figuring an abundance of muscle replaced by inorganic-microspheres (Schultze, 1989, Plate 3, figs. 4-6; Plate 4, figs. 1-2), he offered no explanation for the peculiar crystal habit.

None of the above models is accepted here. Martill's (1988, 1991) and Hirschler *et al's.* (1990a, 1990b) models are rejected due to the lack of any unequivocal evidence for the direct involvement of microbes in the formation of these microspheres (see Section 3.3.1), whilst Allison's (1988d) model, I suggest is more likely to result in a homogeneous distribution of crystallites rather than their concentration in to microspheres.

Instead, the similarity of microspheres replacing the soft tissues of the Romualdo Member biota to spheres of apatite precipitated *in vitro* in the absence of microbes (Lowenstam, 1981), suggests these tissues to have been replaced initially by an amorphous calcium phosphate phase (ACP) which then experienced a period of recrystallization (Wilby, 1993).

The thermodynamics and kinetics of the formation of inorganic HAP microspheres in supersaturated solutions approximating to physiological fluids are relatively well understood. Microspheres develop *in vitro* only when levels of phosphate supersaturation are 'relatively high' (Boskey and Posner, 1976) and the ambient pH is neutral to slightly alkaline. Under such conditions, an unstable ACP phase is precipitated (Eanes *et al.*, 1965; Walton *et al.*, 1967; Termine, 1972) which subsequently transforms via a metastable intermediate phase, octacalcium phosphate (OCP), into poorly crystalline HAP which itself slowly improves in perfection as a result of Ostwald ripening (Eanes and Posner, 1970). The process of ACP crystallization is believed to be an autocatalitic conversion (Eanes and Posner, 1965; and, Boskey and Posner, 1973) involving the solution translocation of Ca^{2+} and PO_4^{3-} from the ACP phase to OCP-like centres which function as epitaxial substrates for OCP precipitation (Young and Brown, 1982). Since each subsequent phase in this progression is more stable than the previous, the conversion of ACP to HAP via OCP is thermodynamically favoured. The emerging crystalline phases grow normal to the surface of the ACP often producing a hollow space (previously occupied by the ACP) surrounded by a rim of HAP crystals - a microsphere.

A delay in the crystallization of HAP (but not the precipitation of ACP) may be induced through the stabilization of the ACP by a number of chemical species even in trace quantities. These include pyrosphosphate and organic phosphonates, nucleotides, acidic proteins, Mg^{2+}, ATP, ADP, certain disphosphates and proteoglycans (see Williams, 1984). It is widely believed that these inhibit transitions either by poisoning the surface of 'protocrystals' or by entering the pre-nuclei HAP structures thereby creating a structural mismatch (Nancollas, 1982, p85).

Microgranular: Martill (1990a, p172) considered granular apatite fabrics to be the result of the direct nucleation of HAP crystallites on to organic matrices. This surmise is supported by the preservation of macromolecular details (Martill, 1990a), and the identification in this study of soft tissues replaced by preferentially aligned crystallites (see Section 4.2.2.1). Such preservation is wholly inconsistent with a period of recrystallization (Wilby, 1993). Briggs and Kear (1993a, figs. 2a, 2c) have been successful in replacing the soft tissues of shrimps (*Palaemon* sp.) with microgranular apatite in the lab.

According to Boskey and Posner (1976), the precipitation of HAP directly from solution without a period of recrystallization occurs only at relatively 'low supersaturations' and when the ambient pH is between 5.6 and 8.5 (Koutsoukos *et al.*, 1980). At such reduced levels of supersaturation, crystal nucleation and growth are extremely slow unless stimulated by the presence of a substrate (i.e. heterogeneous nucleation, *sensu* Mann, 1983, p134-138) such as an organic matrix or earlier mineral phase. This significantly reduces the activation energy for nucleation by reducing the surface free energy (Nancollas, 1979). However, in the presence of inhibitors (as for microspheres, see above), nucleation on to the substrate and growth of the apatite crystallites even under optimum conditions will cease.

3.4 THE RESOLUTION OF SOFT TISSUE PHOSPHATIZATION

The fidelity with which soft tissues are fossilized depends on a number of factors including the style of preservation (see Chapter 4), the replacing microfabric, and the extent of decay prior to phosphatization (see Chapter 6). The resolution of detail typically preserved by each of the major microfabrics described in Section 3.3 is examined here in terms of the

perfection with which they would replace a complex structure which consists essentially of a membrane-bound cuboid containing a number of mutually aligned macromolecules (i.e. a striated muscle sarcomere, text fig. 3.3a).

Microbes: Microbes preserve only a crude replica of the substrate tissue due to their large diameter (many microbes are >1μm) relative to the ultrastructures of metazoan tissues (see Section 3.3.1), their association with degraded tissues, and, their frequent occurrence as coatings (see Chapter 4). Furthermore, in cases of microbial infestation, the substrate tissue itself is not mineralized but rather the microbes are. This immediately reduces the quality of information that is preserved. These factors are compounded by the fact that more often than not, the distribution of the microbes bears no relationship to the structure of the underlying tissue (text fig.3.3b). That is, the dispersal of microbes is usually entirely random (although not always, see Section 5.3).

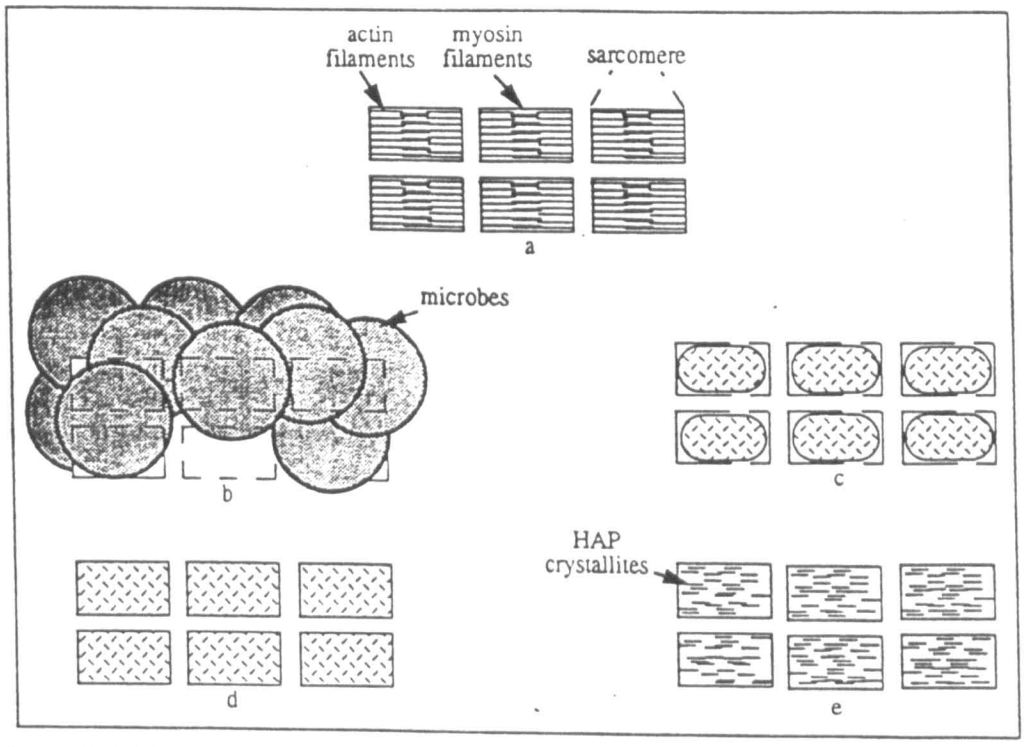
Replacement (and more so coating) of soft tissues by microbes offers little opportunity to examine the tissue's ultrastructure, and in comparison with replacement by inorganic microfabrics (see below), preserves only minimal morphological information.

Inorganic microspheres: Since tissues replaced by microspheres have undergone a period of recrystallization (see Section 3.3.5), they are unlikely to display exceptional ultrastructural details. The quantity of information lost during recrystallization is likely to be exaggerated in cases where ACP was initially precipitated within a confined cuboidal space (e.g. a striated muscle sarcomere). In such cases, the tendency of the evolving HAP crystallites to develop in to spheroidal bodies (i.e. microspheres) will result in the corruption of the tissue's original structure (text fig. 3.3c).

Departure from the original morphology will also be experienced in tissues where dividing membranes are absent. In such cases, an initially even distribution of ACP will lead to the development of a multitude of HAP nuclei whose positions are entirely unrelated to the tissues' original ultrastructure.

Despite the unfavourable period of recrystallization involved in their production, microspheres are still capable of preserving subcellular details. This is a consequence of their

relatively small diameter (usually <1μm), and their frequent substrate-controlled distribution (see fig.4.13).



Text figure 3.3: The resolution of detail preserved by each of the different apatite microfabrics (shading indicates phosphatization). a) The simplified structure of six striated muscle sarcomeres; b) sarcomeres pseudomorphed by microbes of "average" size; c) replacement by inorganic microspheres; d) replacement by inorganic microgranular apatite; e) replacement by structurally orientated HAP crystallites.

Inorganic microgranular (non-spherulitic): Granular apatite microfabrics may replace soft tissues with remarkable precision (see text figure 3.3d). Subcellular resolution is typical, but in the most exceptional cases (see Section 4.2.2.1), macromolecular details may be preserved (Martill, 1990a). This results from the small size of the crystallites (often only 20nm long), and their direct nucleation on to the organic substrates (see text fig. 3.3e). The resolution of detail preserved is dictated only by the size of the replacing crystallites, and their relationship to the substrate (see Section 8.2.3).

A comparison of two identical biological structures, one replaced by microspheres and the other replaced by microgranular apatite, clearly demonstrates the difference in resolution afforded by each of the two inorganic microfabrics (figs. 3.20 and 3.21).

3.4.1 THE FIDELITY OF SOFT TISSUE FOSSILIZATION: A COMPARISON OF DIFFERENT MECHANISMS OF PRESERVATION

Martill (1990b) introduced the use of arbitrary thresholds (e.g. the retention of cellular details, macromolecular resolution etc) as a means of expressing the quality and quantity of information retained by a fossil. These were later refined by Allison and Briggs (1991b) and termed "taphonomic thresholds". Martill (1990b, table 1) utilized taphonomic thresholds to some effect as a means of comparing the fidelity of various mechanisms of soft tissue fossilization based on their most impressive fossil representatives. However, Martill (1990b) only compared a few processes of fossilization and did not distinguish between phosphatized soft tissues which are microbially infested, coated, or permineralized (*sensu* Allison, 1988a, p334).

Martill's (1990b) theme is expanded here to encompass a greater number of lagerstätten and therefore more processes. Each one is expressed as a line whose position relative to various arbitrary levels of resolution, reflects the finesse of the mechanism of soft tissue fossilization (table 3.2).

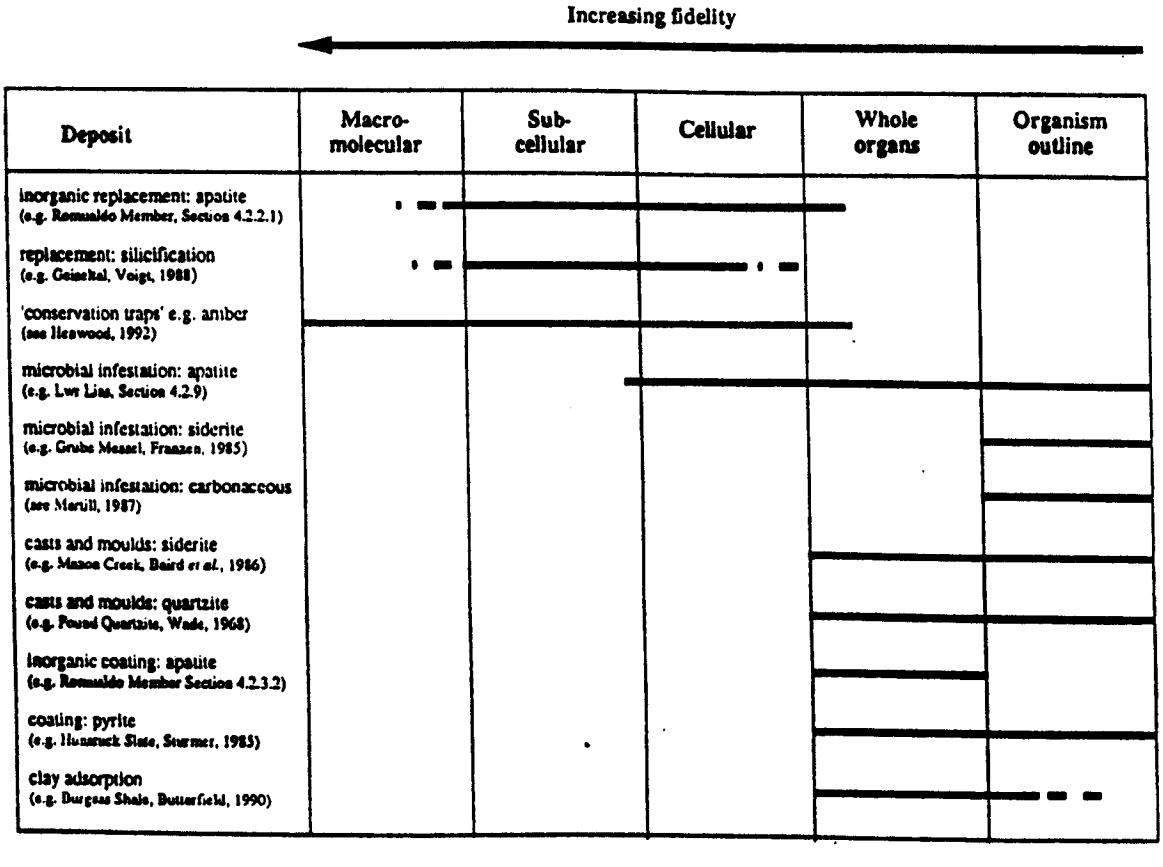


Table 3.2: The fidelity of various mechanisms of soft tissue fossilization

DISCUSSION: It is clear that with the exception of various "conservation traps" (*sensu* Seilacher *et al.*, 1985) such as amber (see Henwood, 1992), peat bogs (see Brothwell, 1987) and permafrost (see Guthrie, 1990), and *perhaps* replacement by silica (see Voigt, 1988), no mechanism of fossilization is capable of preserving more exceptional anatomical detail than when the tissues are permineralized by apatite. For example, most mechanisms of soft tissue fossilization such as microbial infestation (e.g. Grube Messel, Franzen, 1985; Portland Roach, see Section 5.2.4), preservation as casts and moulds (e.g. Mazon Creek, Baird *et al.*, 1986), mineral coatings (e.g. Orsten, Müller, 1985), and clay adsorbtion (e.g. Burgess Shale, Butterfield, 1990) rarely preserve more detail than the tissue's outline and/or gross morphology. In contrast, inorganic replacement of soft tissues by apatite may preserve macromolecular details (Martill, 1990a). Phosphatized soft tissues therefore provide palaeontologists with a tremendous opportunity to examine the biology, physiology, and evolutionary relationships of extinct organisms.

Allison (1988a p334) considered the exceptional preservation of inorganically phosphatized soft tissues over those replaced by other authigenic minerals to be a reflection of the rapidity of apatite precipitation in sedimentary profiles relative to the other mineral phases. This is almost certainly correct but is not necessarily the only factor involved. For example, it is possible that chemically reactive organic substrates and microenvironments created by the initial stages of decay may have a greater affinity for the precipitation of apatite than for other mineral phases. That is, other phases may have been saturated but lacked compatible nucleation sites within the carcasses until decay had progressed further.

An important point to note from table 3.2 is that the mineralogy by which microbes are preserved is of little consequence to the resolution of detailed preserved by microbially infested soft tissues. In all such cases, the finesse of replication is ultimately dictated by the diameter of the microbes, the style of infestation, and the extent of decay prior to mineralization. Therefore, it is not surprising to see that all such deposits 'straddle' similar taphonomic thresholds.

3.5 CONCLUSIONS

1) The soft tissues of the Romualdo Member biota have a hydroxyapatite mineralogy.

2) The soft tissues are replaced by three major groups of microfabrics. These are: mineralized microbes; inorganic precipitates which have experienced recrystallization (i.e. microspheres); and, inorganic crystallites which nucleated directly onto the organic substrates from solution (i.e. microgranular apatite).

3) The distinction between microbial and inorganic microfabrics is genetic, whereas the division of inorganically precipitated phases into microspherulitic and microgranular fabrics reflects differences in the microenvironment at the time of mineralization.

4) The geochemical requirements for microbial and inorganic phosphatization overlap since microfabrics indicative of both mechanisms may occur within individual fossils of the Romualdo Member.

5) Each apatitic microfabric replicates soft tissues with a predictable level of precision. Microbes rarely preserve details finer than the tissue's gross morphology; inorganic microspheres are capable of pseudomorphing subcellular structures; and granular apatite may fossilize macromolecular details.

6) Phosphatized soft tissues probably offer palaeontologists their greatest opportunity to examine the biology, physiology, and evolutionary relationships of extinct organisms.

7) The exceptionally high level of resolution displayed by the soft tissues of the Romualdo Member biota suggests that they have not experienced any late-stage recrystallization events, or compaction.

CHAPTER 4
PHOSPHATIZED SOFT TISSUES OF THE ROMUALDO MEMBER

4.1 INTRODUCTION

Very little is known of the abundance and diversity of phosphatized soft tissues in individual deposits, and even less of any variations in the preservational style of phosphatized soft tissues within and between fossils of the same deposit. Such a knowledge would provide an important step towards an understanding of the controls on soft tissue phosphatization and may reveal tissue- and/or substrate-specific trends in mineralization. The Romualdo Member provides an ideal opportunity for performing such an investigation since phosphatized soft tissues are abundant and have been recorded from a number of taxa (see Bate, 1971; Cressey and Patterson, 1973; Martill, 1988; Martill and Unwin, 1989; Wilby and Martill, 1992). In addition, the tissues are replaced by a variety of microfabrics (see Chapter 3).

Presented below are the results of a detailed investigation of the phosphatized soft tissues of the Romualdo Member biota. This demonstrates the abundance and magnificence of phosphatized soft tissues from this deposit, and serves to demonstrate the palaeobiological potential of such material.

4.2 SOFT TISSUE ATLAS OF THE ROMUALDO MEMBER

The significance of the Romualdo Member as a source of exceptionally well preserved soft tissues was first recognised by Bate (1971, 1972, 1973). He examined more than 180 near-complete specimens of a new genus of ostracode (*Pattersoncypris micropapillosa*) from a single concretion containing a specimen of the teleost fish - *Cladocyclus gardneri* Agassiz. The exquisite preservation of sensory setae, thoracic appendages, the calcite carapace, and male copulatory organs permitted Bate to assess accurately the phylogenetic status of the ostracodes relative to extant representatives, and to interpret their ecology.

Well preserved specimens of a parasitic copepod (*Kabatarina pattersoni*) described from the gill chambers of two specimens of *Cladocyclus gardneri* Agassiz (Cressey and Patterson

1973; and Cressey and Boxshall 1989) further highlighted the potential of the Romualdo Member for yielding rarely fossilized taxa *in their entirety*. These specimens were particularly important finds since they represent the oldest known record of the class Copepoda, and the only fossil parasitic copepods.

Although phosphatized soft tissues were first described from invertebrates (see above), emphasis has subsequently been placed on the fossilized soft tissues of the elopomorph fishes. Martill (1988, 1989a, 1989b, 1990a) has repeatedly demonstrated this material to be exceptionally well preserved. Most notably, he has figured examples of striated muscle (Martill, 1990a, figs. a, b, and d); gills (Martill, 1989b, fig. 9); dermis (Martill, 1990b, text fig. 2e); eggs (Martill, 1989a, Plate 3, fig. e); and stomach walls (Martill, 1989b, fig. 8). The alimentary tract and stomach contents of these fish have also been figured by Wilby and Martill (1992). This work has been stimulated by a desire to understand more fully the processes of soft tissue phosphatization, and by the very real possibility of being able to determine rates of microevolution based on soft tissue anatomy. The diversity of the fauna of the Romualdo Member also provides an opportunity to examine the soft tissues of organisms which are extinct (e.g. dinosaurs, pterosaurs and a variety of 'primitive fish'). This wish was partly realised by the discovery of two, three-dimensionally preserved pterosaur wing membranes (see Campos *et al.*, 1984; Martill and Unwin, 1989; Martill *et al.*, 1990).

As yet no systematic examination of the soft tissues of the Romualdo Member, their taxonomic distribution, or their preservational styles has been published. Presented below is a detailed synthesis of soft tissues recovered from thirty or so concretions from the Romualdo Member. This is not exhaustive; soft tissues never encountered before, and new styles of preservation are identified in nearly every new acid preparation. The figures serve to demonstrate the exceptional nature of the phosphatized soft tissues from this deposit. Emphasis is placed on the tissues and their preservational styles rather than on any taxonomic implications.

4.2.1 PHOSPHATIZED SOFT TISSUES IN HAND SPECIMEN

The ability to identify phosphatized soft tissues in hand specimens of pterosaurs, fish, shrimps and coprolites (see below) has greatly reduced the numbers of 'barren' specimens destroyed unnecessarily. Unfortunately, this is not possible with the microfauna, and therefore the recovery of phosphatized soft tissues from these organisms relies entirely on their fortuitous association with concretions containing macrofossils (e.g. see Bate, 1971; Cressey and Patterson, 1973; Wilby and Martill, 1992).

In hand specimen, phosphatized soft tissues of macrofossils vary from white to buff in colour and are exceedingly friable. In unprepared fossil fish, phosphatized skin is often the only evidence of soft tissue mineralization. This takes the form of thin white lines which outline the posterior edge of the scales. The presence of fossilized skin however, does not necessarily signal the preservation of other organs. The occurrence of internal organs may only be established in those fish in which the scales have been removed during acid digestion, fossil preparation, or during decay. Frequently, when the concretions are split in the field, scales are retained by the counterpart, thus exposing skeletal muscle. On the basis of randomly selected, unprepared material from the eastern extremity of the Romualdo Member's outcrop, Martill (pers. comm. 1992) estimated 20% of all *Rhacolepis* sp. and *Notelops* sp. to contain fossilized muscle. Amazingly, this is almost certainly an underestimate of the actual abundance of phosphatized soft tissues in these taxa. Apparently 'barren' specimens frequently reveal phosphatized soft tissues once acid prepared. Indeed, the present study has demonstrated that nearly 30% of *Rhacolepis* sp. and *Notelops* sp. specimens contain at least some fossilized muscle. Phosphatization is however, far less common in transgressive fish than in comparably sized flat lying fish of the same genera and from the same collection locality.

The quantity of soft tissues preserved in individual fish is extremely variable (see Section 8.2.1 for an explanation). For example, in some specimens of *Rhacolepis* sp. and *Notelops* sp., almost all of the musculature may be preserved, whereas in others, only isolated patches of skeletal muscle or the dorsal fin adductors (or even nothing at all) are preserved. In most specimens, the fossilized muscle is restricted to a thin band (usually less than 6mm) located just beneath the dermis; deeper muscle is only very rarely preserved (see Section 8.2.1 for

an explanation). In flat lying fish, muscle is usually restricted to the lower side of the body (i.e. the side in contact with the sediment), although rarely the muscle on both the upper and lower surfaces may be preserved. Muscle of the caudal peduncle and that located dorsal to the midline is usually preserved *in situ,* whereas that surrounding the body cavity is frequently disorganised or translocated *en mass* away from its original position. Often, disorganised muscle remains in the body cavity as a 'lag' (see fig. 2.5), but it may be transported beyond the fish. Sectioned fish indicate such disruption to be limited entirely to muscle from the upper surface; the lower one remaining completely undisturbed. This suggests the disruption to be associated with the escape of decay gases (see Section 2.5.3) and provides a convincing explanation for the apparent preservation of muscle only on the lower surface of most specimens of flat lying fish. In transgressive fish, skeletal muscle may be displaced anteriorly (along with skeletal elements) as far as the head.

In clean acid digestion residues, the identification of soft tissues is straight-forward. Individual muscle fibres, the secondary lamellae of gills, eggs, and the villi on sections of the alimentary tract may all be identified. Commonly, muscle fibres display a gradational colour change along their length from white, through buff, to deep orange/brown. No corresponding change in gross mineralogy, crystal microfabrics, or density of mineralization is detectable in SEM. The variation therefore probably reflects subtle differences in iron oxide staining, organic content, or the quantity of apatite deposited as a coating on the surface of the tissues.

In thin sections, even at magnifications as low as x40, structures such as stomach wall villi and the yolk of eggs may be distinguished. Variations in the colour of this material (unlike that of acid digested material) relates both to the orientation of the interstitial calcites relative to the plane of polarization, and to the density of soft tissue mineralization (see Martill *et al.*, 1992, and Section 7.2). Translucent tissues are more heavily mineralized than those which are dark brown, which are themselves more pervasively phosphatized than 'brick-red' coloured tissues.

4.2.2 VERTEBRATES

The macrofauna of the Romualdo Member is dominated by vertebrates, and includes actinopterygian and elasmobranch fish (see Maisey, 1991 for a complete review), turtles (Price, 1973), crocodiles (Price, 1959; Kellner, 1987), pterosaurs (see Wellnhofer, 1985; Unwin, 1988; Kellner, 1989; Kellner and Campos, 1990), and a dinosaur (Campos, 1985). Phosphatized soft tissues have thus far only been reported from fish (Martill, 1988) and pterosaurs (Martill and Unwin, 1989). This almost certainly reflects the abundance of these two groups over the other vertebrates.

The abundance of fish in the Romualdo Member far exceeds that of pterosaurs. This study therefore concentrates largely on the soft tissues of the fish.

4.2.2.1 FISH

The ichthyofauna of the Romualdo Member is both abundant and diverse (see Maisey, 1991). Phosphatized soft tissues were first recorded in the fish - *Brannerion vestitum* Jordan and Branner, *Notelops brama* (Agassiz) and *Rhacolepis* sp. - by Martill (1988), and have subsequently been reported from a specimen of *Tribodus limae* (Brito, 1992). Extensive collecting for this study has demonstrated phosphatized soft tissues also to occur in *Rhinobatos* sp., *Vinctifer* sp., *Cladocyclus* sp., and *Tharrias* sp. (table 4.1).

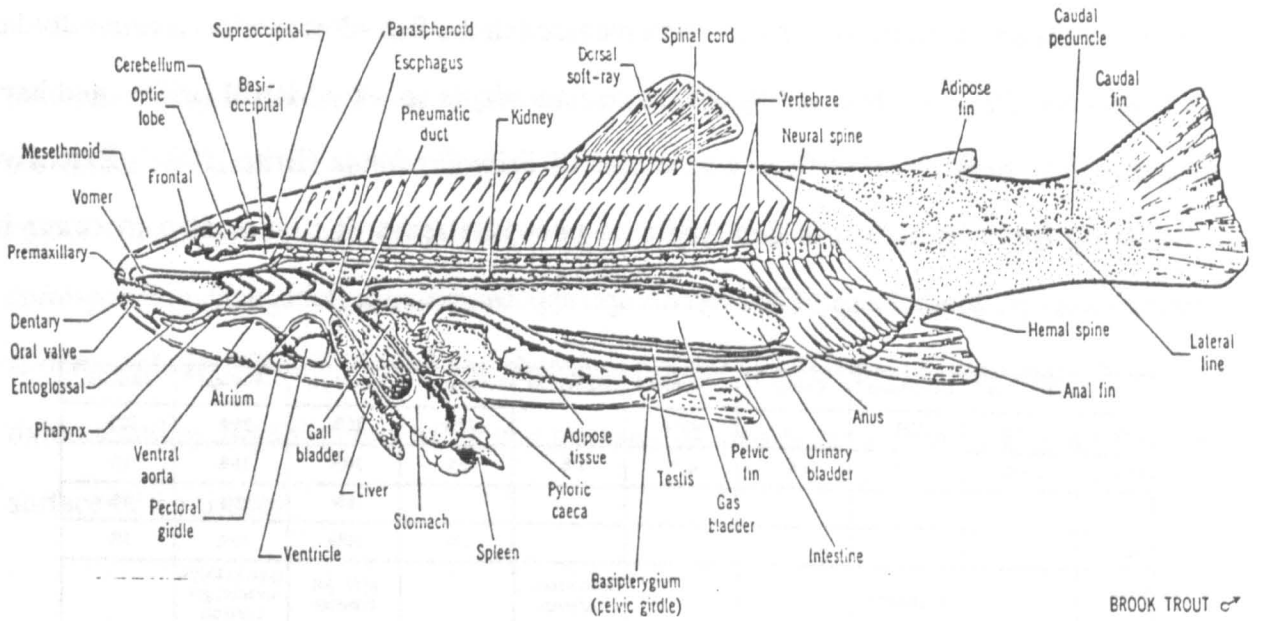
	Tribodus [1]	*Rhinobatos* [2]	*Enneles* [3]	*Vinctifer* [4]	*Cladocyclus* [5]	*Brannerion* [6]	*Rhacolepis* [7]	*Notelops* [8]	*Tharrias* [9]
Dermis	100%	20%		<1%		10%	35%	35%	10%
Muscle	100%	20%	1%	<1%	1%	5%	30%	30%	1%
Gills							15%	15%	5%
Gut						2%	10%	10%	5%
Other		coprolites			blood vessels, parasites		eyes, gut contents	eyes, kidneys, ovaries, gut contents	

Table 4.1: Estimates for the abundance of phosphatized soft tissues in fish genera from the Romualdo Member (data for *Tribodus* taken from Brito, 1992; data for *Enneles* taken from Jordan and Branner, 1908). Number of specimens on which the estimates are based: 1) 1, 2) 10, 3) ≈100, 4) >300, 5) >100, 6) >150, 7) >500, 8) >500, 9) ≈200.

Despite their wide taxonomic distribution, phosphatized soft tissues are only commonly encountered in specimens of *Notelops* sp. (see fig. 2.23), *Rhacolepis* sp. (fig. 4.1), *Brannerion* (see Martill, 1988 plate 1, fig. 1; plate 2, fig. 1), and *Tharrias*. This is almost certainly *partly* a reflection of the relative abundance of these four genera. Due to the rarity of

specimens of *Tribodus* and *Rhinobatos* (I have only examined one specimen of *Tribodus* and ten specimens of *Rhinobatos*) it is not possible at present to comment with certainty on the frequency with which phosphatized soft tissues occur in these two genera. However, initial indications suggest that phosphatized soft tissues are both common and extensively preserved in specimens of both genera (e.g. see Brito, 1992, figs. 3a and b). The scarcity of phosphatized soft tissues in the abundant specimens of *Vinctifer* and *Cladocyclus* however, requires an alternative explanation (see Section 8.2.1).

Unless otherwise stated, the following account refers exclusively to material recovered from specimens of *Notelops* sp. and *Rhacolepis* sp. The extent, distribution, and appearance of phosphatized soft tissues (in hand specimen and in SEM) in a typical specimen of *Rhacolepis* sp. is given in fig. 4.1, and a schematic diagram of fish organization is provided for reference in text fig. 4.1.



Text figure 4.1: The soft tissues anatomy of a soft-rayed bony fish (reproduced from Lagler *et al.*, 1962).

DERMIS: skin is usually only patchily preserved in individual fish but is generally very common. At low magnifications, complex whorls and ridges cover those surfaces which where in contact with the scales. Often, finer details are completely destroyed by microbial infestations (almost exclusively by morphotype 1a microbes), or by the displacive growth of calcite rhombs between adjacent scales (see fig. 2.15). However, when replaced by inorganic microspheres and microgranular apatite, some ultrastructural detail is usually

preserved, and in a few specimens, at least two layers can be identified (fig. 4.2). The most peripheral is usually only 15μm thick and consists of densely packed cells (with a heavily mineralized central nucleus) embedded in an anastomosing, filamentous material. TEM examination reveals the crystallites to be perfectly aligned parallel to the long-axis of these filaments and to be of a remarkably constant size (fig. 4.3). The preferential orientation of the crystallites is confirmed by micro-electron diffraction patterns which display clear groupings of spots into arcs (fig. 4.4) typical of preferentially aligned crystals (Beeston *et al.*, 1973, pp199-200). Comparison with Recent fish skin suggests these filaments to be collagenous plasma membrane tonofibrils which comprise the most peripherally located layer (the fusiform layer) of the epidermis of many fish (Leonard and Summers, 1976). An exteriorly located layer - the mucous coating or cuticular layer - which is well developed in many Recent teleosts, has not been observed in the fossil material.

The second layer - the dermis - is located internally to the epidermis and is usually only ≈50μm thick. It consists of a number of superimposed layers (composed of mutually aligned filaments), each parallel to the surface of the epidermis, whose filaments are orientated differently to those of layers above and below (fig. 4.5). This most closely resembles the stratum compactum of Recent teleosts which consists predominantly of collagen and elastic fibres (Groman, 1982).

Beneath the stratum compactum, the hypodermis is occasionally preserved. This is thin (<1mm) and consists of bands of fibrous connective tissue (fig. 4.6) underlying a smooth membrane.

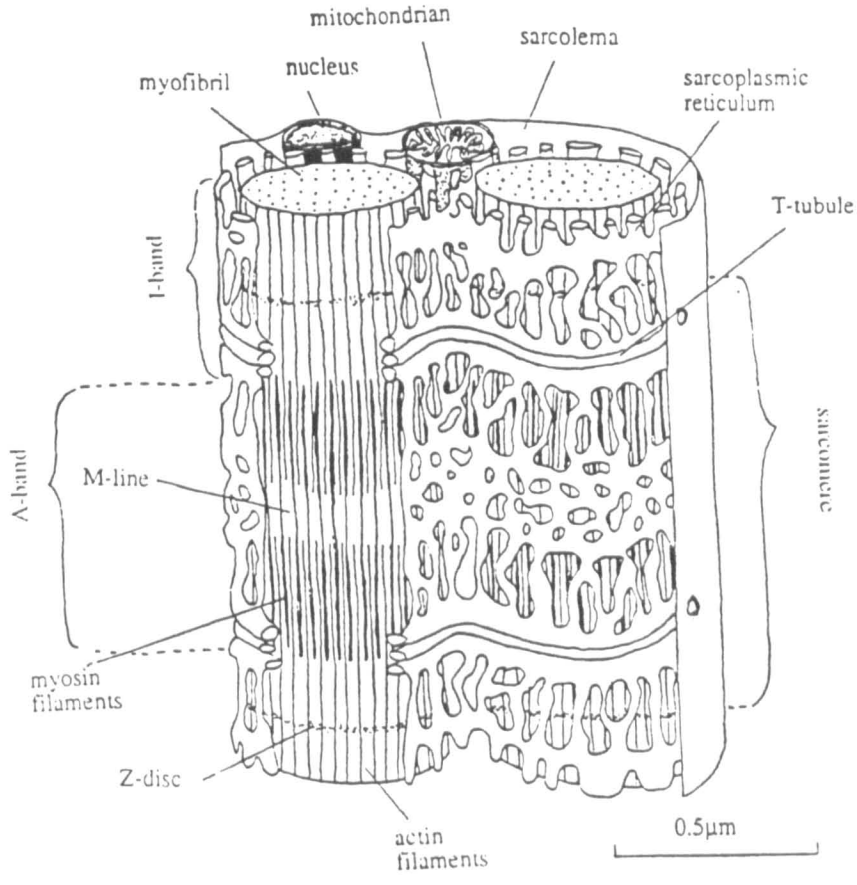
The dermis of *Brannerion* and *Tharrias* similarly display several distinct layers which are comparable in structure and thickness to those described above. Dermis has also been identified in a single specimen of *Rhinobatos* (DM/Santana/36), but this has not been examined in SEM. Even in hand specimen however, it is clear that the dermis of *Rhinobatos* has an entirely different structure to that of *Notelops* and *Rhacolepis*. In particular, it is relatively thin (< 1mm thick) and studded with dermal denticles.

MUSCLE: A variety of muscular tissues are commonly preserved. These include the involuntary smooth muscles of the alimentary tract and arteries (see below); and the striated (or skeletal) locomotory muscles. The occurrence of each type is described separately below:

Striated muscle (see text fig. 4.2 for schematic illustration and Alberts *et al.*, 1989, pp613-624 for a full description): The striated muscle fibres of Recent fish are multinucleate and are composed of hundreds of myofibrils which extend along the entire length of the fibres. Each myofibril is divided along its length into a series of cuboidal sarcomeres which contain actin (thin) and myosin (thick) filaments arranged in regular bands whose position is stabilized by a variety of accessory proteins (see text fig. 6.2). Surrounding each sarcomere is the sarcoplasmic reticulum from which T-tubules extend. It is along these tubules that stimuli are transmitted for muscle contraction. When receiving a signal, the myosin filaments of each sarcomere 'crawl' along the length of the surrounding actin filaments. Since the actin filaments are embedded in a thin sheet of connective tissue (the Z-disc), and the fibres are connected to one another at their terminal ends (by connective filaments across a sheet of connective tissue - the myoseptum), the movement of the actin and myosin filaments against one another causes the entire fibre to contract. The energy required for each contraction is supplied (in the form of ATP) by the abundant numbers of mitochondria which are associated with each myofibril.

Skeletal muscle is by far the most commonly preserved tissue in fish from the Romualdo Member. Both 'red' and 'white' skeletal muscle has been recovered from specimens of *Rhacolepis* sp. and *Notelops* sp.. Red muscle, since it is utilized in sustained periods of activity and therefore requires an abundant source of energy, contains a greater density of mitochondria and blood capillaries than white muscle which is involved in short bursts of fast swimming (Lagler *et al.*, 1962). When viewed in cross-section, the two types of muscle are easily distinguished on the basis of fibre diameter and location (Lagler *et al.*, 1962). Red muscle fibres have a relatively small diameter and are usually confined to a thin strip just beneath the dermis along each fish's lateral line, whilst white muscle fibres make up the bulk of the muscle mass and have a much greater diameter (see fig. 8.1).

Although there are some structural differences between the two types of striated muscle, both are dealt with as one in the following discussion.

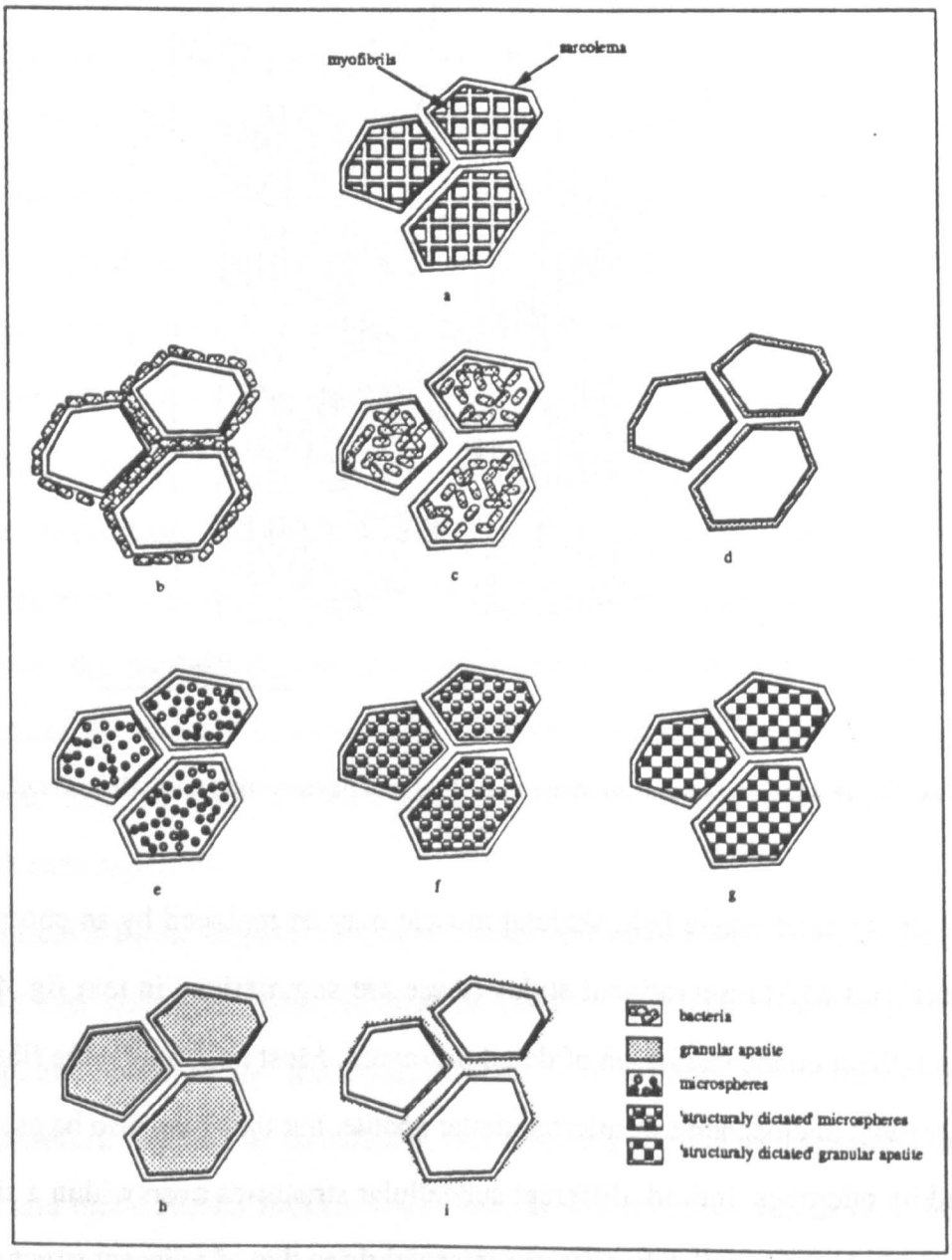


Text Figure 4.2: Schematized structure of striated muscle (modified after Alberts *et al.*, 1989).

Even within a single fish, skeletal muscle may be replaced by an enormous variety of microfabrics and preservational styles (these are summarized in text fig. 4.3). This has a marked effect on the resolution of detail preserved. Most commonly, the fibres are replaced by inorganic microspheres or microgranular apatite, but they may also be pseudomorphed or coated by microbes. Indeed, different subcellular structures even within a single fibre may be preserved by a completely different microfabric to that of adjacent structures. Peculiarly, some structural elements are never, or are only very rarely preserved. These include the mitochondria, blood vessels and micro-capillaries, the Z-discs, sarcolemmas, myosepta, the sarcoplasmic reticulum, and system of T-tubules. Possible causes of such differential mineralization are examined in Section 8.2.3.

Sarcomeres: In the most exceptionally preserved examples (i.e. when replaced by microgranular apatite), the morphology of the fossil skeletal muscle is almost indistinguishable from that of extant fish muscle (compare figs. 4.7 and 6.12). Both

longitudinal and transverse banding patterns may be preserved. These are of comparable dimensions to those of Recent muscle. The longitudinal bands are of a constant thickness (0.7μm) and usually traverse the fibre's entire length. These are interpreted to be myofibrils.



Text figure 4.3: Common preservational styles and replacing microfabrics of skeletal muscle in fish from the Romualdo Member. a) transverse section of original muscle fibres; b) sarcolemmas coated by microbes; c) muscle fibres pseudomorphed by microbes; d) sarcolemmas preserved as internal moulds; e) muscle fibres replaced by inorganic microspheres; f) each sarcomere replaced by an individual inorganic microsphere; g) sarcomeres replaced by structurally orientated crystallites; h) muscle fibres replaced by microgranular apatite; i) sarcolemmas coated by microgranular apatite. Note: any combination of the above may occur together.

Each myofibril is divided along its length (by a series of gaps) into hundreds of cuboidal couplets, each about a micron in length. The dividing gaps may vary considerably in width along the length of an individual myofibril from a fraction of a micron (100nm) to several microns (1-2μm). These bands are interpreted to be the M-lines. The simultaneous development of M-lines with enlarged widths (relative to those of *pristine* Recent muscle) in all the sarcomeres of a single muscle fibre, tends to create a series of stacked sheets each consisting of laterally connected couplets (fig. 4.8). Each couplet is itself bisected by an unmineralized zone (<100nm) which corresponds to the Z-disc. The couplets therefore consist of a Z-disc and two halves of adjacent sarcomeres (see Section 6.2.1.2 for a discussion of the mechanisms of their production).

The clarity of banding in fossil striated muscle depends enormously on the replacing microfabrics. The fibre's ultrastructure is frequently obscured by microbial coatings (text fig. 4.3b and fig. 4.9) or by the deposition of apatite between the sarcolemma and fibre proper (text fig. 4.3d and fig. 4.10). Banding is entirely destroyed if the fibre is pseudomorphed by microbes (text fig. 4.3c). In such cases, little more than the cell's three-dimensional shape is preserved. Similarly, most fibres replaced by inorganic microspheres preserve only limited structural detail (text fig. 4.3e and fig. 4.11) although some transverse banding may be preserved (fig. 4.12). In one such example, each half of every couplet was replaced by a single microsphere (fig. 4.13 and text fig. 4.3f), thereby corrupting the original cuboidal morphology of the sarcomeres. Conversely, replacement by non-spherulitic inorganic microfabrics may preserve the sarcomeres with remarkable precision (text fig. 4.3g and fig. 4.7). With progressive degrees of crystal aggregation (see Section 3.3.4.2) however, the tissue's ultrastructure becomes increasingly corrupted (text fig. 4.3h) until it approaches the levels of resolution typically preserved by inorganic microspheres.

Connective tissues: Z-discs appear never to be phosphatized in striated muscle, although it is of coarse impossible to be certain of this in those fibres which are so 'heavily' mineralized that they display no internal structure. Similarly, myosepta are only rarely preserved. These relatively thick sheets of connective tissue are nearly always pseudomorphed by microbes and therefore display little structure.

In contrast, the connective fibrils protruding from the terminal ends of the fibres are frequently preserved. These are always replaced by microgranular apatite (even when the associated fibre is replaced by a different crystal microfabric), and frequently display a regular periodicity of thickenings (fig. 4.14). The periodic thickenings probably correspond to an underlying structural unit in the substrate biomolecule (see Section 8.3.1).

Sarcolemma: Recent striated muscle fibres are entirely enclosed within a thin plasma membrane - the sarcolemma. These structures are only very rarely fossilized in fish muscle from the Romualdo Member. Most commonly, they are preserved by infesting microbes as external moulds. In such cases, since the sarcolemma itself is not preserved, a gap develops between the muscle fibre and the enshrouding microorganisms (fig. 4.9 and text fig. 4.3b). Occasionally, the muscle fibres are also not mineralized and the infested sarcolemas are preserved as a series of stacked hollow tubes. Martill (1989a, Plate 2, fig. c; and, 1990a, fig. 1c) proposed these structures to be *mineralized* sarcolemas. However, the presence of typical microbial microfabrics and the enlarged thickness of these structures relative to true sarcolemas dismisses this proposal.

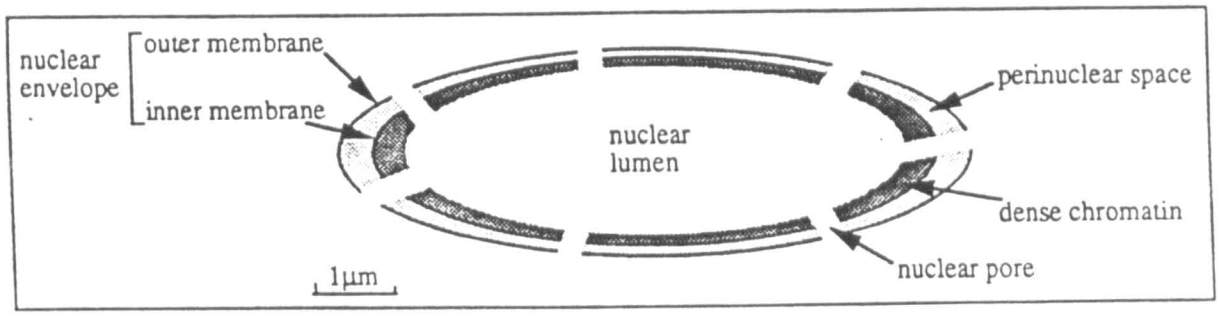
Inorganic replacement of sarcolemas is extremely rare, but has been observed (fig. 4.15). More commonly, they are preserved by inorganic microgranular apatite as an external- (fig. 4.16 and text fig. 4.3i) or internal-mould (text fig. 4.3d).

Nuclei (see text fig. 4.4): Recent striated muscle fibres have hundreds of nuclei dispersed in regular rows across their outer surfaces just beneath the sarcolemma. These are elliptical in shape (see fig. 6.2) and are always orientated with their long axes parallel to that of the muscle fibre (Kessel *et al.*, 1979).

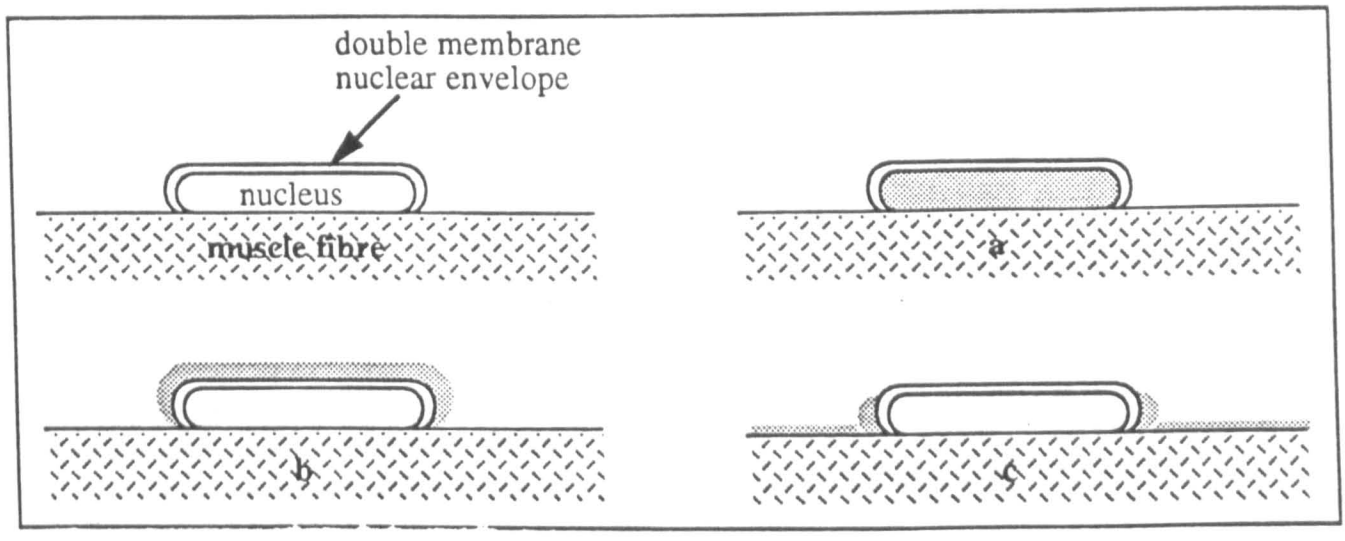
Structures identical to these are frequently preserved on the outer surface of striated muscle fibres in fish from the Romualdo Member. These are of comparable size to Recent nuclei (i.e. ~7x2μm), and are similarly evenly dispersed and orientated parallel to the muscle fibres. Their preservation varies considerably (see text fig. 4.5).

Most are preserved as internal moulds (text fig. 4.5a) either by inorganic microspheres (see fig. 4.13) or by microgranular apatite (fig. 4.17). Elsewhere however, they may be

preserved as external moulds (text fig. 4.5b), or as partially mineralized 'ghosts' (text fig. 4.5c and fig. 4.18) whereby their previous existence is revealed by the presence of an unmineralized 'shadow' which represents their previous point of attachment.



Text figure 4.4: Schematized structure of muscle cell nuclei.

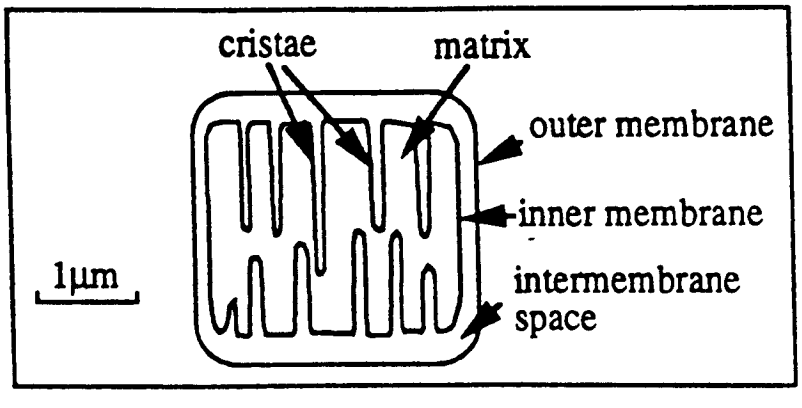


Text figure 4.5: Common preservational styles of muscle cell nuclei. a) internal mould; b) external mould; c) partial external mould or "ghost preservation". Stippled areas represent mineralization.

Mitochondria (see text fig. 4.6): In living striated muscle, mitochondria are abundant and are located both between the myofibrils and on the periphery of each fibre (just beneath the sarcolemma) (Kessel *et al.*, 1979). Each one is roughly spheroidal and approximately 3µm in diameter. The most characteristic feature of mitochondria is their double membrane wall, the internal membrane being intensely folded into cristae (see fig. 6.7).

Similarly sized spheroidal organelles are extremely rare in the fossil striated muscle except in a few samples (always red muscle) where they are exceptionally abundant (fig. 4.19). Most frequently they are preserved as both an internal and an external mould, the

intermembrane space remaining unmineralized (although see fig. 4.20). However, much like nuclei (see above), mitochondria are also preserved as 'ghosts' whereby they protected the underlying myofibrils from being coated by apatite during mineralization and remained themselves unmineralized (fig. 4.21).



Text figure 4.6: Schematized structure of mitochondria.

Only in one case have the cristae been tentatively identified. These were preserved as unmineralized areas between the densely phosphatized matrix (see fig. 4.20).

Extracellular structures: In Recent skeletal muscle (particularly red muscle), a complex network of blood vessels and capillaries criss-crosses each fibre. These ensure the fibre receives a continual supply of metabolites and that waste products are rapidly removed. Such structures are only rarely encountered in the fossil skeletal muscle. However, when they are fossilized, the characteristic distribution of longitudinally aligned capillaries and countersunk transverse capillary loops of Recent muscle (Kessel *et al.*, 1979, p45 and p145) is clear (fig. 4.23). Most commonly, they are preserved as external moulds by inorganic microspheres, although they have also been observed as 'ghosts' on the external surfaces of some fibres (fig. 4.24).

T-tubules and sarcoplasmic reticulum: The T-tubules and sarcoplasmic reticulum of Recent striated muscle are generally only visible in sectioned material (i.e. in TEM). They appear as small (typically 100nm diameter) hollow circles located between the myofibrils (see fig. 6.14). Comparable structures in sectioned fossil muscle are rare. This probably reflects their small size relative to the replacing crystal microfabrics. When they are preserved, they usually occur as non-mineralized cavities (fig. 4.24), although a few mineralized examples have been observed (see fig. 4.18).

CIRCULATORY SYSTEM: Veins and arteries (fig. 4.25) are preserved in abundance in fish from the Romualdo Member. Since they are rarely observed *in situ*, one can only assume them to have originally been associated with the skeletal muscle.

As in Recent examples, the walls of the fossilized veins and arteries (the latter being considerably thickened relative to the former) are differentiated into three layers (Kessel *et al.*, 1979, pp41-52). These with radial distance from the lumen are:

1) *Tunica intima.* This consists of a single layer of simple squamous cells termed the endothelium. These cells are aligned parallel to the vessel's longitudinal axis and form a smooth surface against the lumen.

2) *Tunica media.* The squames of the tunica intima lie on the circularly-arranged smooth muscle and elastic fibres of the tunica media (fig. 4.26). These fibres may be up to 100μm in length but are rarely more than 1μm in diameter. Unlike striated muscle fibres, they are completely structureless when viewed in SEM.

3) *Tunica adventitia.* The tunica adventitia which consists of connective fibres running longitudinally along the vessel (fig. 4.27) and is usually ≈80μm thick.

GILLS: The gills of actinopterygian fish are concerned predominantly with respiration, although they may also be involved in feeding and salt excretion (Lagler *et al.*, 1962). Their primary use as a gas exchanger necessitates the presentation of a large surface area of blood-filled vessels to the water. This is accomplished by the development of a number of paired arteries (gill filaments) into a series of lobate or sack-like structures called secondary lamellae. Each secondary lamella is enclosed by a very thin and permeable membrane which is covered with a single layer of epithelial cells. It is across these that oxygen diffuses into, and carbon dioxide diffuses from the blood. Each gill filament is supported on a bony gill ray extending from the gill arch in such a manner as to form a compact basket over which water is flushed either by expanding and contracting the buccal cavity, or by continually swimming with the mouth agape (i.e. ram ventilation).

Gills are extremely complex and taphonomically delicate structures. Their frequent preservation in three fish taxa (see below) attests to the rapid (Martill and Harper, 1990) and delicate nature of the mineralizing process. In specimens of *Notelops* sp. and *Rhacolepis*

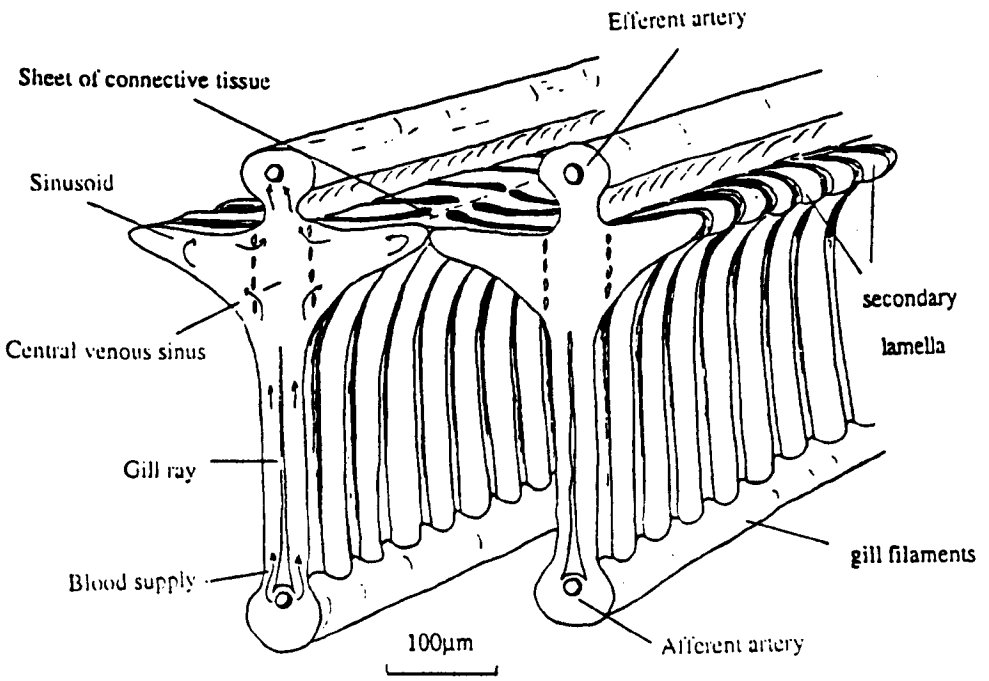
sp., the entire respiratory apparatus may be preserved *in situ*, although it usually breaks up into individual filaments or groups of filaments during acid digestion. The gill apparatus of *Notelops* sp. and *Rhacolepis* sp. are morphologically similar. I therefore treat them together (refer to text fig. 4.7):

Each filament is proximally supported on a blade-like, primarily mineralized bony gill ray. The afferent artery which runs along a groove at the base of the ray is protected, much like the distally located efferent artery (fig. 4.28), by a 50 μm thick epithelium. This is nearly always preserved by microbes as an internal mould of the basal membrane. When the arteries are replaced by cryptocrystalline apatite, longitudinal smooth muscle fibres and connective tissues may be visible (see Martill and Harper, 1990, Plate 1, fig. 4).

The secondary lamellae, where they are attached to the gill rays, are relatively low in relief (little more than 30μm), but distally develop into lobate/tubular projections (150μm long) which point away from the central sinus and efferent artery. Curiously, and unlike idealised gill filaments (see Lagler *et al.*, 1962, figs. 8.1 and 8.4), the projections from each secondary lamellae are superficially fused to adjacent lamellae of the neighbouring filaments along the length of the entire filament (text fig. 4.7). This is afforded by a thin layer of epithelium which runs along the most distal point of each projection. Martill (1989a) and Martill and Harper (1990) considered this to be a blood vessel. However, the secondary lamellae only abut against this feature and do not 'feed' into it (fig. 4.29). Similar structures are described by Muir and Kendall (1968) from Skipjack tuna (*Katsuwonus*), swordfish (*Xiphias*), and bowfin (*Amia*). In the former two, this is an adaptation to strengthen the gas exchanger against damage incurred during ram ventilation and is characteristic of fast swimming fish (Roberts, 1975), whilst in *Amia*, fusion protects the gills during periods of air breathing (Bone and Marshall, 1983). The former adaptation is most consistent with the sleek, fusiform outline of *Notelops brama* Agassiz and *Rhacolepis buccalis* Agassiz.

Detailed three-dimensional reconstructions of the gills are hampered by the presence of a 'blanket' of morphotype 1a microbes which mask much of the tissue's fine details. The correct identification of structures is further hampered by the repetitive breakage of the fused apparatus in a plane normal to the distal tip of each filament's ray (fig. 4.30) so that only the distal portion of the gill apparatus is usually figured (Martill, 1989a, Plate 3, fig. a). On top

of this, the style of preservation varies slightly between individual specimens. This has been demonstrated experimentally by Martill and Harper (1990) to be related to the degree of postmortem degradation that took place prior to phosphatization.



Text figure 4.7: Reconstruction of two gill filaments from the gill apparatus of *Rhacolepis* sp. The filaments are sectioned to illustrate the direction of blood flow (arrowed).

Within the gills, although mineralization is not random (fig. 4.31), a combination of membrane replacements, internal and external microbial coatings, microgranular internal casts, and unmineralized areas, makes identification of specific ultrastructural elements difficult. The central sinus is always preserved as a microgranular internal mould (fig. 4.31), whereas the sinusoid remains largely unmineralized (fig. 4.31 'S'). No evidence exists of the pilaster cells which maintain the diameter of the secondary lamellae in Recent gills. Their absence is almost certainly a taphonomic phenomenon.

In all three specimens of *Tharrias* sp. digested in acid, mineralization of the gills was restricted entirely to the proximal portions of each secondary lamella (i.e. where they are attached to the gill rays). Only remnants of the arteries, epithelium and connective tissues were identified (fig. 4.32). Soft tissues of the gill apparatus of the other fish genera listed in text figure 4.0 have not been observed.

DIGESTIVE SYSTEM: The alimentary canal and its contents are frequently preserved in specimens of *Notelops* sp. and *Rhacolepis* sp. (Wilby and Martill, 1992). Extramural organs (i.e. those associated with the gut) however, except for isolated fragments of smooth muscle from the fishes' gas bladders, have not been observed. The one exception was located with EDAX elemental mapping (see Appendix 3i). This is tentatively identified as a trunk kidney based on its close proximity to the alimentary canal and on the preservation of what appear to be tubules. Phosphatized kidneys have also been reported in fish from the Devonian Cleveland Shale (see Section 5.2.14).

Alimentary tract: Considerable lengths of the alimentary canal may be preserved in any one fish (see fig. 4.0). A variety of tissues may therefore be identified in SEM, each of which corresponds to a different area of the gut (e.g. see Martill, 1988, Plate 3, figs. 2a and b). It is frequently possible to distinguish numerous concentric layers which are directly comparable to those of Recent digestive systems (see Kessel *et al.*, 1979). For example, in some fragments of the ?rectum, five distinct layers may be identified. These with radial distance from the lumen are:

1) *Epithelial cells.* Epithelial cells cover the tongue-like longitudinal primary folds of the lumen and are preserved as solid internal moulds by aggregates of microgranular apatite and (fig. 4.33).

2) *Squamous epithelium.* Interstices between the epithelial cells suggest the squamous epithelium (≈10μm thick) to have extended as far as the lumen, but to have been only patchily preserved. This has resulted in the preservation of a network of mineral loci which are separated by large cavities (see fig. 4.33). In other specimens, the squames are extremely well preserved despite being at least partially pseudomorphed by microbes (fig. 4.34).

3) *Stratum compactum.* The connective tissue of the stratum compactum is heavily mineralized and over 20μm thick (see fig. 4.34). It consists of crystallites of similar size (≈50nm) which are preferentially aligned in to bands and whorls.

4) *Areolar connective tissue.* A thick (>30μm) band of areolar connective tissue is located between the stratum compactum and underlying muscularis externa. The amorphous

ground substance of the areolar tissue is not mineralized, whereas various structures embedded within it are. This has resulted in the whole layer appearing rather spongy. The mineralized structures probably include blood vessels, nerves, connective tissues, and lymphatic vessels. These are preserved both as internal- (granular apatite) and as external-moulds (inorganic microspheres).

5) *Muscularis externa*. This is usually densely mineralized by microgranular apatite. Individual string-like smooth muscle fibres are clearly discernible and inter-dispersed (when viewed in TEM) with circular cavities, 600nm in diameter, which are either isolated or grouped into threes and fours. The identity of these cavities is uncertain, but they may well represent various sections through a system of blood capillaries. The reticulate network of blood vessels and capillaries in the submucosa of another specimen is figured by Wilby and Martill (1992, figs. 7a and b). These have been preferentially infilled by masses of inorganic microspheres which faithfully reproduce the extent of the circulatory system. A similar result may be achieved with Recent tissues by careful resin embedding, and is greatly exploited by biologists (e.g. see Kessel *et al.*, 1979).

The ultrastructure of other areas of the alimentary tract is similarly extremely well preserved. Commonly, their stratified squamous epithelium is also only patchily mineralized, and their epithelial cells are similarly preserved as internal casts by microgranular apatite. In the most spectacular cases, the epithelial cells retain microvilli (fig. 4.35).

Stomach contents: The stomachs of *Rhacolepis* sp. and *Notelops* sp. frequently contain recognisable elements of their last meal. Despite the only partial preservation of the stomach lining in most specimens, food items are usually preserved *in situ*. Shrimps (see Section 4.2.3.2), juvenile fish, and pelagic larval bivalves (see Section 4.2.3.1) may all be preserved in exceptional detail and in numbers well above those of the surrounding sediment (Wilby and Martill, 1992). The ingested fish occur either as masses of disarticulated, well preserved bones, or as fully articulated, three-dimensional skeletons orientated head first with their long axis sub-parallel to that of the predator fish (see Wilby and Martill, 1992, fig. 3). In marked contrast to the host fish, the soft tissues of the prey fish are never preserved.

This anomaly is presumably due to the rapid digestion of the prey's soft tissues such that they were unavailable to be mineralized. Nevertheless, it is clear that the alimentary tracts of fish in the Romualdo Member provide a valuable source of material for dietary- and ontogenetic-studies, as well as a site for the preservation of otherwise poorly represented fossil material.

Rhacolepis and *Notelops* were evidently opportunistic predators, feeding on any small mid-water pelagic organisms. Predator/prey relationships have also been noted by Maisey (1991). He described a specimen of *Cladocyclus* (AMNH 2983) in which an entire *Rhacolepis* had been ingested tail-first, and a *Enneles* which contained a head-first *Vinctifer* in its pharynx. The authenticity of these specimens however, has recently been questioned by some workers (pers. comm. Martill, 1992).

The occurrence of stomach contents within vertebrate fossils from other deposits has been widely documented (for a review see Pollard, 1990). The preservation of exceptionally well preserved soft tissues in such microenvironments however, has only previously been recorded in two instances, both of which refer to phosphatized ostracodes. The first was recorded from a coprolite in the Wealden beds of Belgium (Bertrand, 1903), whilst the second came from the stomach of a pliosaur in the Upper Jurassic (Early Volgian) of the Saratov district (USSR) of the Volga River (Dzik, 1978). The abundance, and exceptional nature of prey items in the alimentary tracts of predatory fish in the Romualdo Member suggests prospecting in the guts of vertebrates from other deposits may be rewarding.

Cololites (*sensu* Agassiz, 1838): A large proportion (perhaps over 50%) of specimens of *Rhacolepis* sp. and *Notelops* sp. contain at least some phosphatized material within their alimentary tract. Posterior to the stomach, this is usually amorphous and consists of dense aggregates of mineralized microbes and inorganic microspheres. Similar *in situ* coprolitic material has been described in fish from various other deposits (e.g. see Agassiz, 1838; Häntzschel *et al.*, 1968). In particular, Clark (1989) has described phosphatized bacteria from the alimentary tract of a conodontophagous palaeoniscid fish from the Ardross shrimp bed (Fife), which are remarkably similar to morphotype 6 microbes of the Romualdo Member (see Section 3.3.2).

Coprolites: Isolated, three-dimensional coprolites are exceedingly common in concretions from the Romualdo Member. These vary considerably in form reflecting the taxonomic diversity of the fauna and probably also differences in consistency of the faecal matter (see Edwards, 1976). All are pseudomorphed by a combination of microbes and inorganic microspheres.

The largest coprolite encountered (14cm x 3cm), a spiral form, has relatively blunt terminal ends and lacks visible organic inclusions. Internally, it is composed of primary and secondary folds (separated from one another presumably by an unmineralized mucous membrane) such that the entire structure forms a single intensely folded flat sheet of faeces (fig. 4.36). The outermost whorl is parted from those inside along much of its length, and in some places has exfoliated. Similar uncoiling has been recorded from the faeces of the Australian lungfish - *Neoceratodus fosteri* - (Jain, 1983).

Spiral coprolites of similar morphology have been widely reported from other deposits (see for example Zangerl and Richardson, 1963; Jain, 1983; and, Schmitz, 1991). There has however, been considerable debate as to whether these represent true coprolites or fossilized spiral valves (see Duffin, 1979). In the present case, evidence of uncoiling, the absence of associated skeletal or soft tissue elements from the parent organism, and a complete lack of prey items, suggests this material to have been fully digested and expelled prior to mineralization. It is therefore probably a true coprolite.

The parent organism of this coprolite is difficult to establish. According to Fange and Grove (1979), only elasmobranchs, 'holosteans', and coelacanths possess a valvular intestine capable of producing such a coprolite. Six genera in the Romualdo Member are therefore possible candidates. These are: *Tribodus*, *Rhinobatos*, *Obaichthys*, *Enneles*, *Axelrodichthys*, and, *Mawsonia*. The small size of known specimens of the first two named genera preclude them as likely candidates, but beyond this, it is impossible to discriminate any further.

The commonest coprolite in the Romualdo Member consists of a simple, blunt-ended cylinder whose dimensions may range from 7mm x 15mm to 15mm x 50mm. The frequency

with which this form occurs, and their similarity to certain coprolites described from other deposits (see Edwards, 1976), suggests them to have been derived from fish.

Similar coprolites but of considerably smaller size are commonly encountered in acid residues. These consist of a single, simple cylinder 80μm in diameter and up to 3mm long which is folded back on itself several times. Fish fry are favoured over epifaunal invertebrates as the source of these coprolites due to the rarity of epifaunal invertebrates in the Romualdo Member. The severe folding and breakage of these coprolites may then be tied to their descent through the water column and subsequent impact with the sediment (see Edwards, 1976).

Gastric residues: Gastric residues represent the indigestible remnants of ingested prey. Most frequently they take the form of spheroidal, tightly packed bodies, with or without interstitial amorphous coprolitic material (Zangerl and Richardson, 1963). Similar packages, some of which are cemented by phosphatic material indistinguishable from that of cololites, are common in the Romualdo Member (Wilby and Martill, 1992). These are 2-6mm in maximum dimension and consist of extremely tightly packed, disarticulated and frequently fractured remnants of shrimp carapaces (fig. 4.37) and/or fish skeletons. The shrimp material regularly display signs of etching and/or bite marks. When not cemented by apatite, the debris is usually loosely cemented by pyrite framboids or limonite (after pyrite).

Frequently, isolated crustacean exoskeletons are also recovered from acid residues. These are not considered to be exuviae.

OTHER TISSUES: Considering the complexity of fish anatomy (see text fig. 4.1), it is important to note that with the exception of those tissues described above and the rare occurrences of eyes and ovaries (see below), no other fossilized soft tissues have been encountered (see Section 8.2.2 for a discussion).

Eyes have been observed *in situ* in one specimen each of *Notelops* sp. (PRW/22) and *Rhacolepis* sp. (PRW/36). These are heavily mineralized, and were therefore not suitable for SEM examination.

Ovaries containing eggs have been identified only on one occasion and then only in thin section with the aid of EDAX analyses (fig. 4.38). These were intimately associated with the posterior portion of the alimentary canal. Each of the eggs (≈150μm diameter) was preserved as an external mould and displayed some internal signs of structure.

4.2.2.2 PTEROSAURS

Pterosaurs are numerically the most abundantly preserved tetrapods in the Romualdo Member (Kellner, 1989; Wellnhofer, 1985, 1991; Kellner and Campos, 1990) and are also taxonomically diverse. Over twelve species (seven genera) have been described, although this is most probably inflated (Unwin, 1988). Two reports of soft tissues have thus far been made, both of which are wing membranes. The first (Campos *et al.*, 1984) gave few details of the tissue's histology, whilst Martill and Unwin (1989) and Martill *et al.* (1990) described a second specimen in considerable detail. This therefore requires no further deliberation here, other than to state that the style of phosphatization is indistinguishable from that of the fish. For example, transverse banding in the striated muscle of the specimen described by Martill *et al.* (1990) is preserved by cryptocrystalline apatite as a series of 'stacked discs' (fig. 4.39) in a manner identical to that encountered in many fish (see fig. 4.8).

4.2.3 INVERTEBRATES

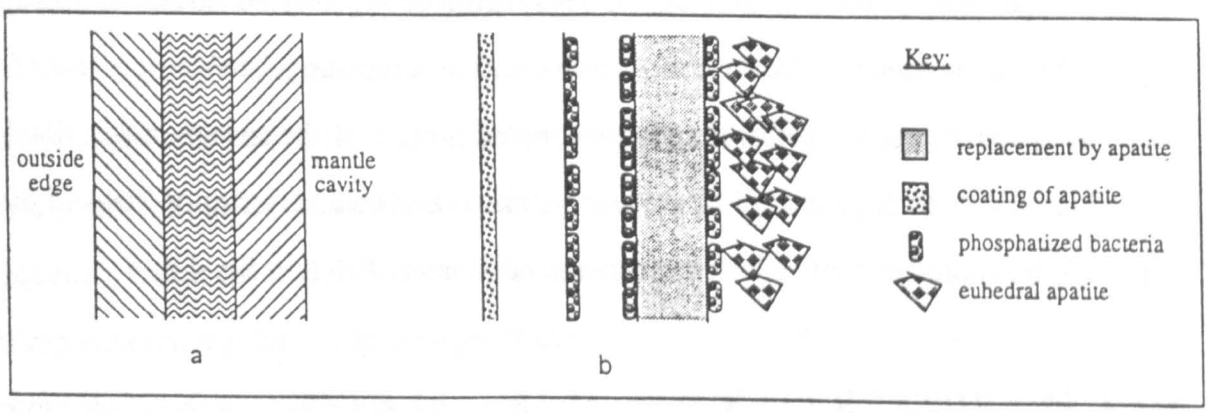
The invertebrate fauna of the Romualdo Member is relatively impoverished. This is attributable to both environmental factors and taphonomic filtering, especially the early diagenetic removal of aragonite (see Section 2.5.1). However, some of the most remarkable secondarily phosphatized tissues are associated with these organisms.

4.2.3.1 MOLLUSCA

GASTROPODA: Soft tissues have not been identified in gastropods. However, the test of one juvenile gastropod was preserved by phosphatized microbes (morphotype 1a) as an internal mould. Fragments of the inner-most layer of the shell were replaced by granular apatite but did not display the characteristic leaf morphology of nacreous aragonite (Bandel, 1991). This style of mouldic preservation is similar to that of many phosphorites

(see for example Manheim *et al.*, 1975) and in particular the winnowed bone-bed at the very base of the Santana Formation (see Section 2.4.6).

BIVALVES: Phosphatized bivalve conchs have been recovered from the stomachs of a single specimen of *Rhacolepis* (PRW/6). Most are preserved by microbes and inorganic microspheres as internal and/or external moulds, although some are partially replaced by microgranular apatite (fig. 4.40). A complex history of valve dissolution, apatite deposition, and microbial infestation has led to the development of an intricate arrangement of replacements and internal and external moulds of the bivalves (summarised in text fig. 4.8). This style of preservation closely resembles that exhibited by Crustacea (see Section 4.2.3.2).



Text figure 4.8: The preservational history of bivalves from the stomach of a *Rhacolepis* sp. (PRW/6). a) origional trilamellar bivalve shell; b) dissolution of middle layer and infestation by microbes; replacement of inner layer; coating of outer shell surface; dissolution of outer shell layer, and growth of euhedral apatite within the mantle cavity.

The small size of the bivalves (<0.5mm) and their occurrence in the alimentary tracts of fishes from sediments in which a shelly fauna is rare, suggests them to be a planktonic larval stage.

4.2.3.2 CRUSTACEA

Crustacea (particularly ostracodes) are ubiquitous in the Romualdo Member, and examples with secondarily phosphatized soft tissues have been widely reported. Bate (1971,

1972, 1973) described phosphatized ostracode eggs, a nearly complete pre-adult series of instars, and adult ostracodes in which the carapace, appendages and various organs were exquisitely preserved. Similarly, entire specimens of a parasitic copepod have been described by Cressey and Patterson (1973) and Cressey and Boxshall (1989).

Even within individuals of the copepods, the exoskeleton shows signs of replacement (Cressey and Boxshall, 1989, Plate 5, fig. 30), internal and external coatings (Cressey and Boxshall, 1989, Plate 4, fig. 21), and microbial infestation (Cressey and Boxshall, 1989, Plate 7, fig. 38). Similarly, the ostracodes are preserved by a complex combination of several preservational styles. Generally, the body and appendages are replaced by microgranular apatite, whilst the external surface of the carapace is coated by apatite, and the space between the carapace and body is infested by microbes (fig. 4.41). The carapace itself is entirely permineralized by apatite, but whether or not the original crystal structure is faithfully reproduced requires further investigation.

DECAPOD SHRIMPS: Well preserved decapod shrimps were first recorded from the Chapada do Araripe by Beurlen (1963). The present study has demonstrated that these are relatively abundant at certain horizons in the Romualdo Member, particularly towards the far western end of the Chapada. Although somewhat crushed (presumably due to decay, see Briggs and Kear, 1993; Zangerl, 1971), the carapace, abdomen, telson and some appendages remain articulated. Fractured surfaces reveal that in some specimens, the gross morphology of at least part of the musculature is preserved by microcrystalline apatite. This suggests investigations of other well preserved Crustacea from the Romualdo Member (e.g. Anostraca, see Maisey, 1991) may be rewarding.

The greatest abundance and opportunity for examining the soft tissue anatomy of Crustacea is however in the stomachs of small predatory fish (Wilby and Martill, 1992). These microenvironments have yielded tens of shrimps in various states of disarticulation and digestion. Many are still fully articulated although most display some degree of degradation. A clear progression in the state of maceration can be recorded in individuals from a single stomach, from nearly pristine specimens (fig. 4.42), to those in which most of the appendages and portions of the exoskeleton are lost (fig. 4.43), and finally, those in

which little anatomical detail is preserved. The absence of the most distal portions of appendages even in specimens with preserved musculature, and the occurrence of appendages as isolated elements, is consistent with much of this damage having been incurred during predation. This is supported by the frequent occurrence of bite marks which have usually been enlarged by exposure to the gastric juices.

Details of the shrimps' appendages are particularly well preserved. These include compound eyes in which individual lenses are perfectly replaced by microgranular apatite (fig. 4.44), antennal scales, exopods with linear rows of setae (fig. 4.45), chelicerae (fig. 4.46), endopods, and maxillipods. Differences in the morphology of the uropods, spinosity of the carapace, and in the length of the rostrum, suggests a number of distinct taxa are preserved. These are probably all new species, but further examination is required for confirmation.

The exoskeleton and various appendages of these shrimps are preserved by a curious combination of microbial infestation, inorganic coatings, and inorganic replacements. This, together with the nonmineralization of specific layers of their multilayered cuticles (see Dalingwater and Mutvei, 1991 for a summary of the structure of crustacean cuticles), can result in an extremely complex cross-section profile (fig. 4.47). Distinguishing between inorganic microspheres and permineralized spherical microorganisms is frequently extremely difficult, particularly when they abut against the carapace or occur within a confined space. In general, the carapace of most shrimps appear to be replaced by inorganically precipitated apatite, although microbes commonly occur as coatings of secondary importance. Most commonly, the cuticle is coated (internally and/or externally) by a thin (600nm) deposit of microgranular apatite or inorganic microspheres (fig. 4.48). Replacement of the cuticle by the same two fabrics has also been observed.

Very often, the most external layer of the exoskeleton (epicuticle) is only patchily replaced whereas those below are either not phosphatized at all (?the calcified layer), or are extensively mineralized by granular apatite (see fig. 4.47). It is not clear whether this is: 1) a reflection of variations in the cuticle's original composition and therefore differences in the speeds at which each layer disintegrates in the gastric juices; 2) the result of variations in each layer's affinity for apatite; or, 3) a combination of both of the previous factors.

The internal projections of the cuticle surrounding the alimentary canal are extremely well preserved in most specimens, such that no replacement microfabrics are visible. This is probably the original chitinous material.

The musculature, ligaments, and associated 'non-structural' soft tissues (i.e. all tissues excluding the carapace) of these shrimps are perhaps the most exceptionally preserved of any tissues in the Romualdo Member. Transverse sections at the abdomen/carapace junction indicate the musculature to be commonly preserved almost in its entirety (fig. 4.49). Often, the sites of muscle anchorage onto the internal surface of detached portions of the carapace are preserved as discrete sub-hexagonal areas of relief. Strikingly similar structures have been recorded from taphonomic experiments by Briggs and Kear (1993a). The most impressive preservation however, is displayed by the sub-cuticular epithelial cells and basal membrane. Frequently, the cells are preserved along the specimen's entire length as a series of juxtaposed hexagons, approximately 15μm in diameter with a centrally located nucleus (fig. 4.50). Both the nuclei and plasma membrane are preserved by microgranular aggregates of apatite as internal moulds (fig. 4.51). The basal membrane may be replaced either by microgranular apatite (fig. 4.51), or by a loosely connected collection of inorganic microspheres (fig. 4.52).

4.3 CONCLUSIONS

1) Phosphatized soft tissues are *exceedingly* abundant in the Romualdo Member. They occur in several groups of organisms which have a poor fossil record, and in at least one group (the pterosaurs) for which there are no extant representatives. The Romualdo Member is therefore of considerable palaeobiological importance.

2) Fish from the Romualdo Member are characterised by the preservation of only a thin peripheral zone of soft tissues. Internally located organs (except for the alimentary tract) are only very rarely preserved.

3) In the fish, mineralized microbes are generally restricted to the alimentary tract and those tissues situated towards the exterior of the carcasses. Microbial microfabrics are much more important in the preservation of soft tissues in the crustaceans.

4) The alimentary tracts of vertebrates have enormous potential for preserving rare taxa in exceptional detail and at relatively high concentrations.

5) The resolution of detail preserved depends on the style of preservation and the replacing microfabric. Preferential alignment of crystallites in some tissues suggests macromolecular details are occasionally preserved; subcellular resolution however is the norm. The most exceptionally well preserved material is not especially scarce, and is frequently sufficiently well preserved to permit one to comment on specific histological details.

6) Phosphatization of soft tissues is taxon-, tissue-, and biomolecule-specific. Some organisms, tissues, and subcellular bodies are never preserved.

7) Within an individual carcass, a single type of tissue may be preserved by a number of different preservational styles and microfabrics.

8) The soft tissues of different groups of organisms are preserved by a different combination of microfabrics and styles of preservation.

CHAPTER 5
TAXONOMIC TRENDS IN THE PRESERVATIONAL STYLE OF PHOSPHATIZED SOFT TISSUES

5.1 INTRODUCTION

The phosphatized soft tissues of only a few deposits have have received detailed systematic attention (e.g. the Alum Shale Formation, Müller, 1985; the Cordillera de Domeyko, Schultze, 1989; and the Romualdo Member, Martill, 1988), and many have not been examined since before the widespread availability of SEMs. This chapter describes (in stratigraphical order) the preservational style(s) and crystal microfabrics of phosphatized soft tissues from fifteen lagerstätten. Material has been examined directly from the Haqel Basin, the Crato Formation, the Portland Roach, the Solnhofen Limestone, the Oxford Clay, the Lower Lias, the Granton shrimp bed, the Gullane shrimp bed, and the Glencartholm Volcanic Beds. My descriptions of soft tissues from deposits for which material was not available to study are based entirely on published records.

I do not intend this chapter to provide a detailed review of the sedimentology and palaeontology of each deposit. Indeed, only a very limited amount of data is available for some, and in certain cases it has not even been possible to establish the formation in which the soft tissues occur. Instead, this chapter concentrates on taxon- and tissue-related trends in the preservational style and microfabrics of phosphatized soft tissues. These are summarized in two classification systems; one based on preservational style, and the other on the fidelity of preservation.

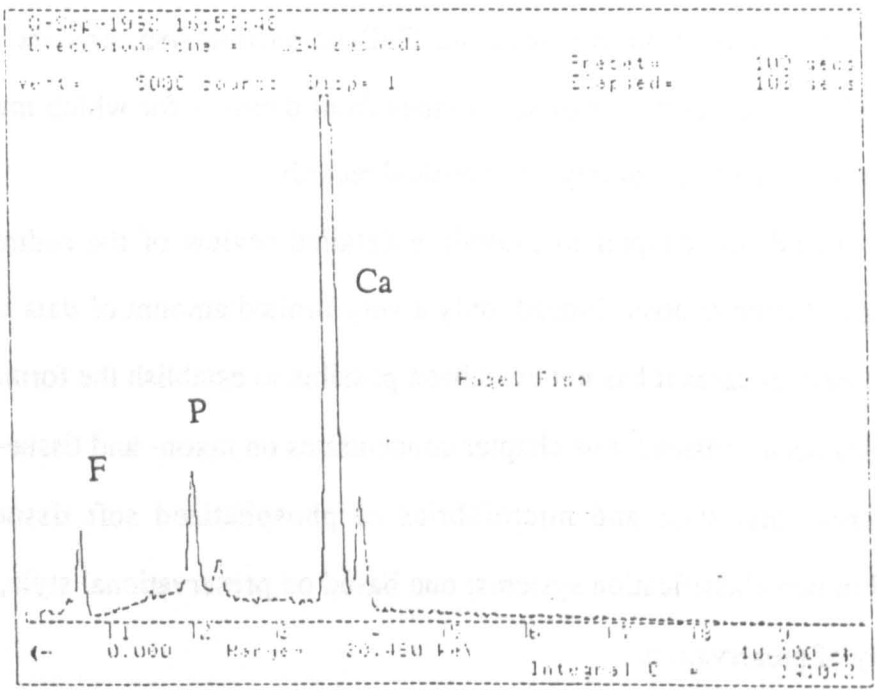
5.2.1 ECKFELD MAARLAKE (Eocene), Eifel, Germany.

Eckfeld Maarlake contains a fossiliferous series of non-marine oilshales. Phosphatized soft tissues have thus far been recorded only from percoid fish (Micklich and Wuttke, 1988). Being pseudomorphed entirely by dense communities of microbes (Micklich and Wuttke, 1988, fig. 8) referable to morphotype 6 microbes of the Romualdo Member (see Section 3.3.2), the fossilized soft tissues preserve no ultrastructural details.

5.2.2 HAQEL BASIN (Lower Cenomanian, Cretaceous), Lebanon.

The lithographic limestones of the Haqel Basin were deposited in small (only a few hundred metres wide) pull-apart structures related to the near by Jordan Rift System (Hückel, 1969). They contain an extremely diverse, predominantly marine fauna which was preserved under an anoxic halocline (Hückel, 1970). Fish, echinoderms, cephalopods, bivalves, gastropods, insects, and worms have been reported (Roger, 1946). Inter-fingered with, and occasionally cross-cutting these sediments are carbonate turbidites.

Phosphatized soft tissues (probably fluorapatite, see text fig. 5.1) are known only from specimens of the fish - *Scombroclupea macrophthalma* - from the lithographic limestones. Only the skeletal muscle *appears* to be preserved. This occurs along the entire length of the fish as a thin (1-2mm), structureless, cream-coloured mass. The myotomes on the upper and lower surfaces of the fish (which almost exclusively lie on their sides) are compounded into a single mass. This is almost certainly a taphonomic phenomenon (see Zangerl, 1971).



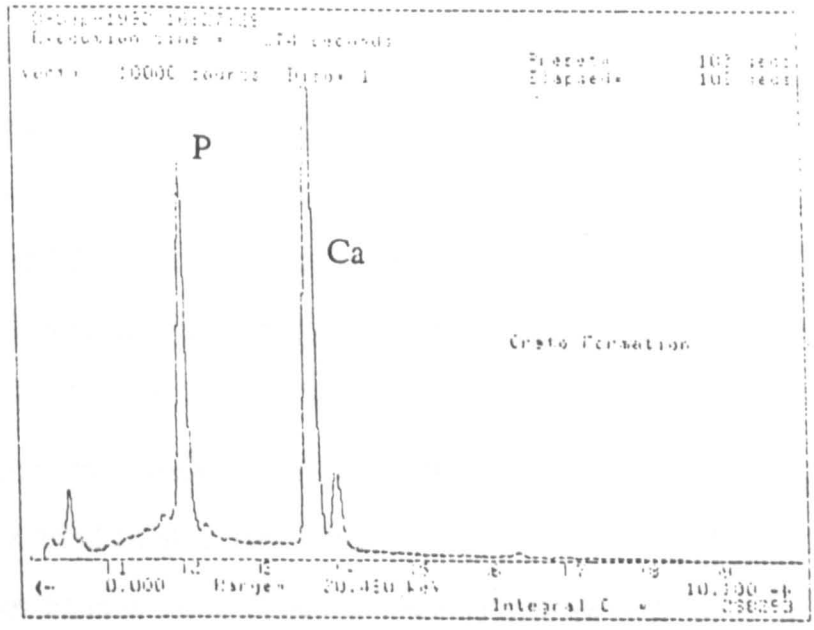
Text figure 5.1: EDAX elemental analysis of soft tissues from *Scombroclupea macrophthalma* (BMNH P4747) from the Haqel lithographic limestones. The elemental peaks are consistent with a fluorapatite composition.

The muscle is completely structureless in SEM and is replaced by a combination of microbes (reminiscent of morphotype 2 microbes of the Romualdo Member, see Section 3.3.2) and inorganic microcrystalline apatite (fig. 5.1). The relative proportions of these two microfabrics varies considerably between different samples, but microbes dominate in most.

5.2.3 THE CRATO FORMATION (Upper Aptian - Lower Albian, Cretaceous), Chapada do Araripe, NE Brazil.

The pyrite-rich plattenkalks and micrites of the Crato Formation were deposited in a restricted lagoon bounded to the north and south by major lineaments (see Section 2.2). The lagoon experienced large influxes of both fresh and marine waters, but for the majority of the time was hypersaline (see Section 2.4.4.2 for a more detailed palaeoenvironmental reconstruction). Intolerable and rapidly fluctuating salinities, and the presence of an anoxic hypolimnion, prevented the lagoon from being permanently populated by a macrofauna. Nevertheless, the Crato Formation contains an abundant, extremely diverse, and exceptionally well preserved allochthonous entomofauna (for a review see Grimaldi, 1990, and Martill, in prep.)(see fig. 2.2) and flora (see Crane and Maisey, 1991 for a review)(see fig. 2.3). Furthermore, juveniles of the gonorhynchiform fish - *Dastilbe elongatus* (see fig. 2.4) - occur at a number of horizons as mass mortalities. These ?freshwater fish were washed into the hypersaline lagoon from bordering river systems by floods (see Section 2.4.4.2).

Both the insects and plants of the Crato Formation are typically replaced by goethite (Grimaldi, 1990). This is a weathering product after pyrite (see Section 2.4.4.1). Soft tissues may however, also be phosphatized. I have recovered phosphatized skeletal muscle (text fig. 5.2) from a single specimen of *Dastilbe* (PRW/17), and Dr. Martill (University of Leicester) has informed me of similarly preserved muscle in an insect.



Text figure 5.2: EDAX elemental analysis of soft tissues from *Dastilbe* sp. (PRW/17) from the Crato Formation. The elemental peaks are consistent with a fluorapatite composition.

As in fish from the Haqel Basin (see Section 5.2.2), taphonomic collapse (see Zangerl, 1971) has resulted in the superimposition of the soft tissues of the upper and lower surfaces of the *Dastilbe*. Skeletal muscle is the only recognisable soft tissue. This is preserved as a single, thin (<1mm), white 'sheet' along the entire length of the specimen. In SEM, little ultrastructural detail is discernible, and in places, the muscle is completely homogenised by the displacive growth of calcite rhombs. Occasionally, however, the outline of individual fibres are preserved as external moulds (fig. 5.2). The character of the replacing crystal microfabric(s) is not clear (probably due to later recrystallization and/or compaction), but mouldic preservation of the muscle fibres (fig. 5.2) suggests microbial infestation to be the dominant preservational mechanism.

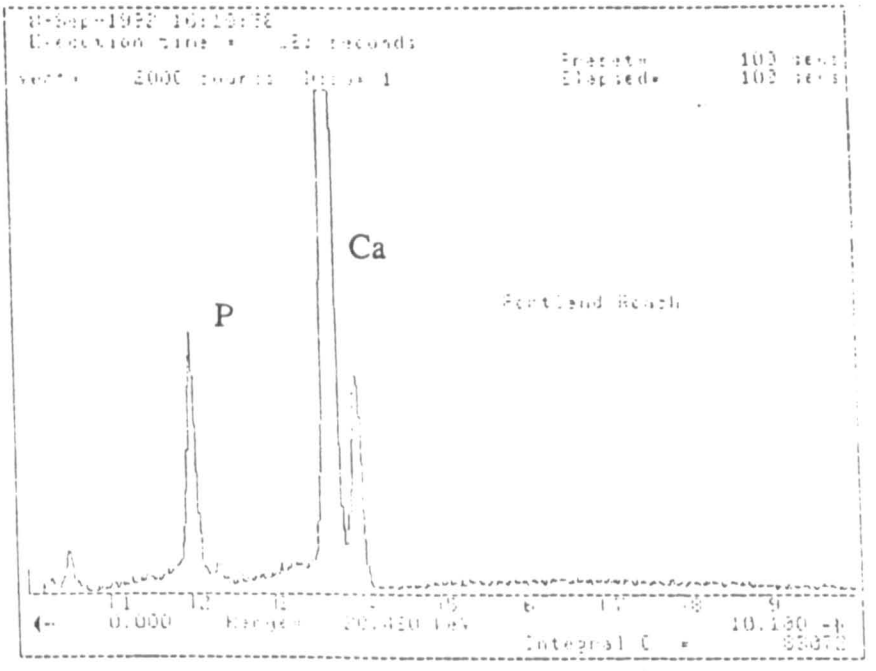
5.2.4 THE PORTLAND ROACH (Upper Jurassic), Dorset.

The Portland Roach is a distinctive cream coloured, oolitic limestone lithology containing winnowed shell beds which appears at several levels in the Winspit Member of the Portland Limestone Formation (Upper Jurassic) (Townson, 1975). It was deposited in a shallow, high energy, marine environment, spotted with patch reefs and oolite banks (Townson, 1975; Hallam and Sellwood, 1976). Articulated internal moulds of the bivalves *Protocardia dissimilis, Myophorella incurva,* and *Laevitrigonia gibbosa,* and the gastropod *Aptyxiella portlandica* are particularly abundant.

Soft tissues (presumed to be silicified) were first identified in specimens of *L. gibbosa* from this lithology by Etheldreda Benett (see Mantell, 1854, p41). However, little is known of the precise collection locality due to the transportation of the Benett Collection to Philadelphia. Recently however, the highest Roach Bed at a number of localities along the south coast of Dorset has yielded fossilized soft tissues (pers. comm. Dr. Whyte, Sheffield University). The lateral extent over which this material occurs remains to be established.

Contrary to previous reports (de la Beche, 1848; Mantell, 1854), the fossilized soft tissues are replaced by apatite (text fig. 5.3). Collecting at King Barrow Quarry, Isle of Portland (Grid Ref. 695720 sheet no. 342), suggests that at least here, soft tissues are extremely abundant. Sixty five percent (sample size=17) of articulated specimens of *L. gibbosa* contained some phosphatized soft tissues, although in only 25% were they

abundant. Productive specimens may sometimes be identified prior to acid digestion by the presence of *in situ* gill support structures (which would otherwise have disarticulated), and/or pits where the phosphatized adductor muscles have been preferentially weathered out. In many specimens however, the soft tissues are no longer *in situ*. In such cases, they frequently form geopetal fills at the base of the internal moulds.



Text figure 5.3: EDAX elemental analysis of soft tissues from L. *gibbosa* (Residue 148) from the Portland Roach. The elemental peaks are consistent with a hydroxyapatite composition.

Phosphatized soft tissues also occur in specimens of *Myophorella incurva*. No phosphatized soft tissues were recovered from one acid digested specimen of *Protocardia dissimilis* and several specimens of *Aptyxiella portlandica*.

A number of tissues may be distinguished in any one specimen of *L. gibbosa* including the gills, the mantle (fig. 5.3), and portions of the ?alimentary tract (fig. 5.4). Frequently ultrastructural details are preserved including ?individual cells, and myofibrils (fig. 5.5). The quality of preservation however, is considerably lower than that of soft tissues from the Romualdo Member. This reflects the fact that the soft tissues of the Portland Roach are infested by microbes, whereas those from the Romualdo Member are predominantly replaced by inorganic microfabrics (see Chapter 4).

Three microbe morphotypes dominate the preservation of soft tissues in the Portland Roach. The commonest - broadly referable to morphotype 4 of the Romualdo Member (see Section 3.3.2) - is extremely well developed (fig. 5.6). In contrast to examples of

morphotype 4 microbes in the Romualdo Member which contain a smooth, membrane-bound central body, those from the Portland Roach contain a much more irregularly shaped internal structure. This suggests the internal structure to be preserved as an external mould rather than as an internal mould (as is the case in specimens from the Romualdo Member).

The second commonest microbe (fig. 5.7) is reminiscent of morphotype 2 microbes of the Romualdo Member (see Section 3.3.2). This occasionally pseudomorphs ultrastructural details such as the myofibrils of muscle fibres, but in most cases, preserves the tissues only as internal and/or external moulds.

The third and rarest morphotype encountered, is indistinguishable from morphotype 1b of the Romualdo Member (see Section 3.3.2). Even the thin cylindrical incisions so characteristic of this morphotype are abundant (fig. 5.8). However, in contrast to the Romualdo Member, the distribution of this microbe in the soft tissues of the Portland Roach is dictated at least partially by ultrastructural features. Thus, myofibrils are occasionally preserved in muscle fibres (see fig. 5.5).

Ooliths trapped within the mantle cavities of the trigoniids are similarly preserved as external moulds by microbes (fig. 5.9); none have been inorganically or microbially *replaced* by apatite. The ooliths presumably entered the mantle cavities of the trigoniids (prior or at the point of death) whilst still calcitic, and were subsequently infested by mineralizing microbes. Remarkably similar phosphatized external moulds of 'ooids' have been figured by Dahanayake and Krumbein (1985, Fig. 4a-c) from the Djebel-Onk phosphorites of the Algeria/Tunisia border. These too were phosphatized whilst enveloped by organic material (in this case stromatolites).

The preservation of muscle in the mantle of *L. gibbosa* and *M. incurva* closely resembles that of muscle from the Crato Formation (see Section 5.2.3) and the Oxford Clay Formation (see Section 5.2.7). The preservational style of these tissues exemplifies a mechanism of phosphatization markedly different to that of the Romualdo Member (i.e. microbial verses inorganic mineralization, see Sections 3.3.3 and 3.3.5 respectively).

5.2.5 THE SOLNHOFEN LIMESTONE (Tithonian, Upper Jurassic), Germany.

The Solnhofen Lithographic Limestone has played a pivotal role in the study of fossil lagerstätten (e.g. see Seilacher *et al.*, 1985; Barthel *et al.*, 1990). It was deposited in a series of back-reef basins whose water columns were probably stratified by a halocline (Keupp, 1977). Autochthonous benthos is absent, the fauna (of over 600 species) being dominated by laterally imported, vagile, littoral benthos and nektonic elements (see Barthel *et al.*, 1990).

Phosphatized soft tissues (see Hewitt and Whyte, 1990 for a discussion of the mineralogy) have been reported from fish, squid, and crustaceans of the Solnhofen Limestone (Reis, 1888, 1893, 1895, 1898). Reis (op. cit.) intimated an inorganic mechanism for their mineralization (see Section 1.6.1). This is supported to some extent by the exceptionally well preserved squid musculature figured by Mehl (1990, Fig.7), and the fish muscle figured by Schweizer (1964, Plate 10, figs. 6-8). On the basis of material examined from other deposits and in particular the Romualdo Member (see Section 3.4), it is clear that microbes are incapable of replacing soft tissues with this level of precision.

However, Mehl (1990) identified bacteria as a major constituent of the gills of the squid. The importance of microbes to the preservation of some soft tissues in the Solnhofen Limestone is confirmed by the present study. The tentacles and mantle of a specimen (BMNH C46871) of the squid - *Plesioteuthis prisca* (Rüppel 1829) are pseudomorphed by microbes resembling morphotype 4 microbes of the Romualdo Member (see Section 3.3.2), although in BMNH C46871 they impinge on one another to a greater extent (fig. 5.10). Although three dimensionally pseudomorphed by the microbes, ultrastructural details are rarely preserved (fig. 5.11).

Some muscle from the tentacles of BMNH C46871 however, is also replaced by inorganic microspheres (fig. 5.12).

Thus, separate tissues within individual organisms, and the same tissue within different organisms would appear to be preserved in the Solnhofen Limestone (as in the Romualdo Member, see Chapter 4) by different microfabrics.

5.2.6 CORDILLERA de DOMEYKO (Oxfordian, Upper Jurassic), Cordillera de Domeyko, N. Chile.

The Oxfordian of the Cordillera de Domeyko, N. Chile consists of a sequence of black sandy marine shales containing carbonate concretions and intercalations of fine grained calcareous sandstone (Chong, 1977). The concretions contain an abundant fauna consisting of crustaceans (Chong and Förster, 1976), fish (see Schultze, 1989, pp186-187 for a review), ammonites (Arratia *et al.*, 1975), teuthoids, bivalves, *Lingula* sp., crocodiles, and ichthyosaurs in that order of abundance (Schultze, 1989). Chong and Förster (1976) interpreted the sediment surface to have been anoxic, whereas Schultze (1989, p187) re-interpreted the depositional environment as being rather more hospitable. He (Schultze, 1989, p187) considered the redox boundary to have been situated within the sediment, and the rather diverse benthic and epibenthic fauna to have lived amongst the fronds of branched algae.

Phosphatized soft tissues have thus far only been recorded from the fish (Schultze, 1989). According to Schultze (1989), fossilized soft tissues including skeletal muscle, blood vessels, swim bladders, dermis, and intestines are exceedingly abundant; nearly every fish containing some material.

The resolution of detail and the style of preservation is remarkably similar to that of material from the Romualdo Member. For example, although the dermis is frequently preserved in fish from both deposits, the outer layer of the epidermis has not been recorded in either. Similarly, skeletal muscle is by far the most commonly preserved tissue in both lagerstätten, but myosepta are not preserved in either (or are at least only very rarely preserved in the Romualdo Member). Even more remarkable are the similarities in the replacing microfabrics. In both deposits, muscle fibres may be replaced by inorganic microspheres whilst their nuclei are pseudomorphed by microcrystalline apatite. Indeed, muscle replaced by inorganic microspheres in the Chilean fish (see Schultze, 1989, Plate 3, fig. 6) is indistinguishable from that of the Romualdo Member (see fig. 4.8).

As in the Romualdo Member, the majority of soft tissues from the Cordillera de Domeyko appear to be replaced by inorganic microfabrics. However, the preservation of some muscle by larger spheres such that no ultrastructural details are visible (Schultze, 1989, p193),

suggests (contrary to Schultze, 1989) that microbes may also have played a role in their fossilization.

5.2.7 THE OXFORD CLAY, CHRISTIAN MALFORD (Athleta Zone, Callovian, Upper Jurassic), Christian Malford, Wiltshire.

Exceptionally well preserved coleoids were first recorded from the Lower Oxford Clay at Christian Malford by Pearce (1842), and have recently been re-examined by Allison (1988d) and Donovan and Crane (1992). Unfortunately, there is no record of the distribution of these and other faunal elements at outcrop. It has therefore not been possible to establish the precise bed or laminae from which they are derived, or to reconstruct accurately the palaeoecology (although the palaeoecology of the Lower Oxford Clay in general has been reviewed by Duff, 1975, and Hudson *et al.*, 1991). However, it is clear that they are preserved with an impoverished benthic fauna in a fissile, bituminous shale which was initially relatively 'soupy' in consistency (Morris, 1979) and probably dysoxic (Wignall and Hallam, 1991).

Laterally equivalent beds have yielded elements of the same coleoid fauna but these lack soft tissues (Mantell, 1848; Carreck, 1960). Phosphatized soft tissues therefore appear to be restricted largely to the 'type' locality, although phosphatized adductor muscles are known from bivalves of another Oxford Clay locality somewhat higher in the sequence (pers. comm. Dr. Harper, Cambridge University).

Phosphatized soft tissues at Christian Malford are restricted entirely to three coleoid taxa: *Belemnotheutis antiquus* Pearce, 1847, *Mastigophora brevipinnus* Owen, and *Romaniteuthis* sp. Typically, mineralization is localized (most commonly to the mantle) and the coleoids are fragmented. However, in some cases, the tentacles complete with suckers and hooks, the mantle, the fins, and the ink sac are all three-dimensionally preserved (see Donovan and Crane, 1992; and, Page and Doyle, 1992, Plate 31, fig. 2, and Plate 32, fig. 2).

Allison (1988d, p405) stated the muscle of the coleoids to be replaced by "filamentous strings of microspherical apatite *which* in places... also occur in aggregated masses" (my italics). These structures are remarkably similar to morphotype 4 microbes of the Romualdo

Member (see Section 3.3.2), although they are more intimately associated with one another (Allison, 1988d, Fig. 5c-e) and more precisely pseudomorph the tissue's original structure.

Examination of material from a specimen of *Belemnotheutis antiquus* Pearce, 1847 (BMNH C46898), confirms that in places the muscle fibres are pseudomorphed by microbes (fig. 5.13) as proposed by Allison (1988d). However, in the majority of muscle that I examined, the fibres were preserved as *external moulds* by the infesting microbes and formed a series of stacked hollow tubes (fig. 5.14)(much like those from the Portland Roach, see Section 5.2.4 and fig. 5.3). Relative to those figured by Allison (1988d, figs. 5c-e), the microorganisms preserving the soft tissues of BMNH C46898 had a slightly smoother external surface, and contrary to the strict definition of morphotype 4 microbes (see Section 3.3.2), many were internally connected (fig. 5.13).

Allison (1988d) discussed the merits of three possible mechanisms of preserving these tissues (see Section 1.6.2). The present study favours "Bacterial fixation" and suggests (as in the Romualdo Member) that the tissues are replaced by more than one microbe.

5.2.8 THE LOMBARDISCHE KIESELKALK FORMATION

(Sinemurian, Lower Jurassic), Osteno, N. Italy.

The Lombardische Kieselkalk Formation contains a 4m thick, marine, spongolithic micrite of extremely limited lateral extent in which the soft tissues of Coleoidea, Crustacea, nematodes, polychaete worms, and Enteropneusta are preserved (Pinna, 1985). Pinna (1985) considered this unit to represent an isolated sedimentary episode in which the bottom waters of the basin became poorly oxygenated and inhabited by a low diversity fauna. Infaunal organisms are absent, suggesting the H_2S-O_2 boundary to have been situated slightly above the sediment/water interface (Pinna, 1985, p180).

The most exceptionally well preserved phosphatized soft tissues are associated with the Thylacocephala (Crustacea) and Coleoidea. In the former, the muscles and branchiae are preserved, whilst in the latter, the arms and traces of muscle occur. Pinna (1985, p177) considered these tissues to have been replaced "molecule for molecule" by amorphous calcium phosphate. However, comparison of this material with phosphatized soft tissues from other lagerstätten and microfabrics preserved in phosphorites, suggests a microbial

origin to be more likely. As in material described above from the Solnhofen Limestone and the Lower Oxford Clay (see Sections 5.2.5 and 5.2.7 respectively), little more than the gross morphology of the muscle fibres is preserved (Pinna, 1985, Plate 1, fig. 9, and Plate 2, figs. 12 and 13). Each fibre is pseudomorphed by a collection of densely packed, polyhedral structures with a mammillate morphology which most closely resembles that of morphotype 2 microbes from the Romualdo Member (see Section 3.3.2). However, relative to the microbes from the Romualdo Member, the spherical structures figured by Pinna (1985, Plate 1, fig. 9, and Plate 2, figs. 12 and 13) are considerably more tightly packed. Very similar crystal aggregate of certain microbial origin have been figured by Soudry and Lewy (1988, Plate 1, figs. d and e) from Lower Cretaceous phosphorites of the Mishash Formation, Israel.

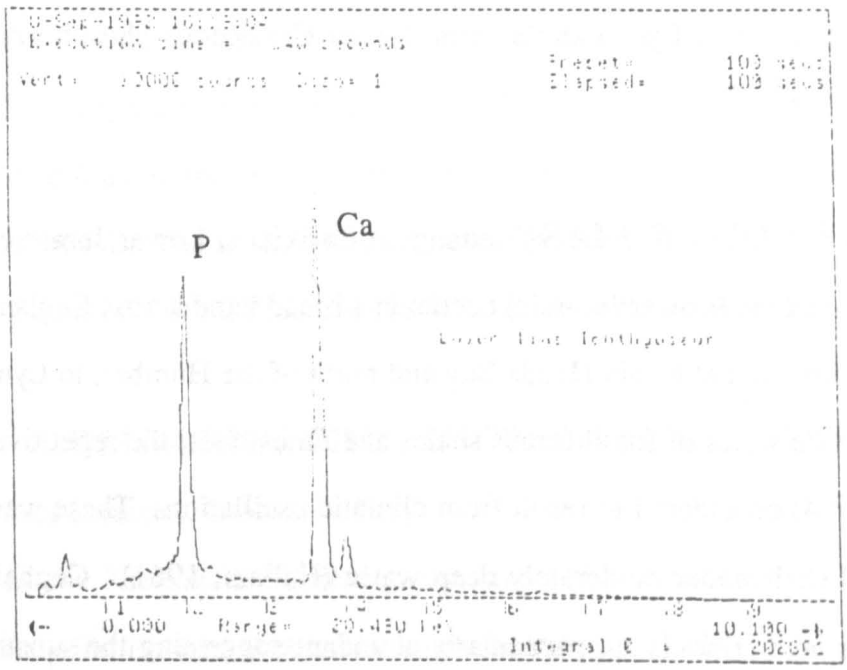
5.2.9 THE LOWER LIAS (Hettangian-Carixians, Lower Jurassic), England.

The Lower Lias (Lower Jurassic) occurs in a broad band across England stretching from the Yorkshire coast at Robin Hoods Bay and north of the Humber, to Lyme Bay in Dorset. It consists of a series of fossiliferous shales and limestones, the repetitive nature of which Hallam (1964) considered to result from climatic oscillations. These were deposited on a continental shelf under moderately deep water (Hallam, 1961). Cephalopods, bivalves, crustaceans, and crinoids are particularly abundant suggesting the substrate to have been relatively stable and oxygenated. Fish, ichthyosaurs, insects, and plants are also recorded (see Hallam, 1961 and Martin *et al.*, 1986 for a review of the stratigraphy and palaeontology of the Lower Lias in Dorset and Leicestershire respectively).

Fossilized soft tissues occur at a number of localities (although the precise horizon is frequently not known) in the Lower Lias including Somerset (Chaning Pearce, 1846), Leicestershire (Martin *et al.*, 1986), and Gloucestershire (Delaire, 1966). These are most frequently associated with ichthyosaurs (see Martin *et al.*, 1986 for a review) and coleoids. The style of preservation is not clear in all recorded cases, but the soft tissues of ichthyosaurs from Barrow-upon-Soar (Leicestershire) are certainly replaced by apatite (Martin *et al.*, 1986). Individual muscle fibres and/or connective fibrils are preserved in many specimens from this region (e.g. LEICS G406.1889 and LEICS G448.1891). These,

according to Martin *et al.* (1986, p65), were smothered by bacterial- or fungal-mats which then subsequently experienced mineralization, and thus preserved the tissues in some detail.

EDAX analysis of the soft tissues of an unidentified juvenile ichthyosaur (DM/Lias/2) from the Lower Lias of Lyme Regis, confirms a phosphate mineralogy for this specimen too (text fig. 5.4). The fossilized soft tissues consist of a thin layer (≈1mm) of cream coloured, structureless material containing impressions of the vertebrae. This lies directly in contact with the sediment. It is not clear wether this represents only the mineralized dermis of the ichthyosaur, or the thoroughly decomposed remnants of both the dermis and skeletal musculature.



Text figure 5.4: EDAX elemental analysis of soft tissues from an ichthyosaur (DM/Lias/2) from the Lower Lias of Dorset. The elemental peaks are consistent with a hydroxyapatite composition.

In contrast to the tissues of ichthyosaurs from Barrow-upon-Soar, the soft tissues of DM/Lias/2 are entirely structureless and thoroughly decomposed; a fact attested by the abundance of 'microbial pits' (fig. 5.15) and mineralized microbes. The tissues are pseudomorphed by two morphotypes. Individuals of the most common one are 2µm in diameter and consist of a thin outer layer (external coating), and a central solid core (internal mould) which is connected to neighbouring cells (fig. 5.16). These most closely resemble morphotype 5 microbes of the Romualdo Member (see Section 3.3.2). The second morphotype is identical to morphotype 6 of the Romualdo Member (see Section 3.3.2).

5.2.10 LAKE ODERNHEIM (Lower Permian), Saar-Nahe Basin, SW Germany.

The sediments of Lake Odernheim consist of a series of rhythmically laminated, non-marine shales which are periodically interrupted by beds cemented by early diagenetic dolomites (Boy and Hartkopf, 1983). The dolomites reflect periods of reduced clastic input, increased evaporation, and the presence of a stratified (chemically and/or thermally) alkalisaline water column with an anaerobic hypolimnion. Each dolomite displays rhythmic increases in organic content which correspond to periods of increasing salinity and the death of planktonic plants which inhabited the surface waters of the lake. These also correspond to the laminae at which fossil amphibians occur (Willems and Wuttke, 1987).

Phosphatized soft tissues have been recorded only from the amphibians of Lake Odernheim (Willems and Wuttke, 1987). Usually, only their skin is preserved, but occasionally muscle fibres are also fossilized (see Willems and Wuttke, 1987, Plate 4, figs. 1-4). The muscle is pseudomorphed with some precision by a combination of microbes (see Willems and Wuttke, 1987, figs. 3 and 4) similar to morphotype 6 microbes of the Romualdo Member (see Section 3.3.2), and microgranular apatite (see Willems and Wuttke, 1987, Plate 4, figs. 5 and 6).

Willems and Wuttke (1987) considered phosphatization to have been stimulated by the infesting microbes, and to have occurred at pH 7-9. To permit a sufficient supply of Ca^{2+} to infiltrate the carcasses from the water column, they (Willems and Wuttke, 1987) envisaged mineralization to have proceeded over a period of several weeks. Willems and Wuttke (1987) proposed this substantial interval between death and mineralization accounted for the preservation of only the most decay-resistant soft tissues (i.e. skin).

5.2.11 THE GRANTON SHRIMP BED (Dinantian, Lower Carboniferous), Edinburgh, Scotland.

The Granton shrimp-bed (Lower Oil Shale Group) is an important member of a series of shrimp-bearing successions in northern Britain which were deposited in coastal delta-plain and interdistributary bay settings of transitional salinity (Cater *et al.*, 1989). The Granton shrimp bed occurs within a ≈100m thick sequence of oil shales which overlie a thick sandstone unit (Briggs and Clarkson, 1983; Cater *et al.*, 1989). This records the

abandonment of a delta lobe (Cater, 1987). The shrimp-bed itself consists of 14-20cm of alternating dark, organic-rich (mostly algal, Briggs and Clarkson, 1983, p162), and light, dolomitic laminae. Cater (1987) interpreted these to represent the low water stand of a stagnant, brackish water lagoon that probably experienced both marine incursions and periods of exposure.

The Granton shrimp bed is famous for yielding the first conodont with soft parts (Briggs *et al.*, 1983). Recently, other enigmatic soft bodied organisms have been discovered including a chordate (Briggs and Clarkson, 1987a), the first recorded fossil tomopterid (a polychaete)(Briggs and Clarkson, 1987b), and hydroids (Briggs and Clarkson, 1983). The fauna however, is dominated by Crustacea, and in particular by *Waterstonella*, *Crangopsis*, and *Palaemysis* (see Briggs *et al.*, 1991 for a detailed review of the fauna). These were killed in mass mortality events probably resulting from a reduction in the oxygen content of the water column following algal blooming (Briggs *et al.*, 1991, p82).

Phosphatized soft tissues have been recorded from all of the fore mentioned organisms (Briggs and Clarkson, 1983). Although structural details such as myotomes, fins and axial traces are visible in specimens of the conodont animal (Briggs *et al.*, 1983), examination of several specimens of *Waterstonella* failed to reveal any evidence of structure in their soft tissues. The former position of the internal organs is recorded only by a thin, structureless film of microgranular apatite, presumably the result of tissue collapse (see Zangerl, 1971) and/or sedimentary compaction.

5.2.12 GULLANE SHRIMP BED (Dinantian, Lower Carboniferous), Lothian, Scotland.

The Gullane shrimp bed (Lower Oil Shale Group) forms part of a contemporaneous series of crustacean communities (which includes the Granton shrimp bed, see Section 5.2.11), each with a slightly different palaeoecology (Schram, 1981; Cater *et al.*, 1989), which were deposited in a delta-plain setting. The stratigraphy of the 12cm thick Gullane shrimp bed has been described in detail by Hesselbo and Trewin (1984). It consists of alternating dolomitic and organic-rich laminae which were deposited in a thermally stratified, fresh water lake or brackish lagoon (Hesselbo and Trewin, 1984). The shrimp bed contains a restricted fauna

consisting almost exclusively of *Tealliocaris* (a shrimp) but with rare occurrences of estuarine fish (Traquair, 1907), hydroids and scorpions. In the sequence at Gullane, the shrimps occur only in the shrimp bed itself. These were probably asphyxiated by algal blooms or lake overturn (Hesselbo and Trewin, 1984).

The preservation of *Tealliocaris* has been discussed in detail by Briggs and Clarkson (1985). They reported the infilling of the exoskeleton by fluorapatite and figured a specimen in which probable impressions of muscle fibres were preserved (Briggs and Clarkson, 1985, fig. 9d). In contrast to the crustaceans of the Granton shrimp bed (see Section 5.2.11), most specimens of *Tealliocaris* are preserved with considerable relief, suggesting phosphatization occurred prior to tissue collapse and sedimentary compaction (Cater *et al.*, 1989 p14). SEM examination of the soft tissues indicates that the longitudinal outline of muscle fibres are occasionally preserved (fig. 5.17). These are pseudomorphed by microfabrics similar to morphotype 4 microbes of the Romualdo Member (see Section 3.3.2). In many cases however, the soft tissues are entirely structureless and the carapace is merely infilled by aggregates of microgranular apatite.

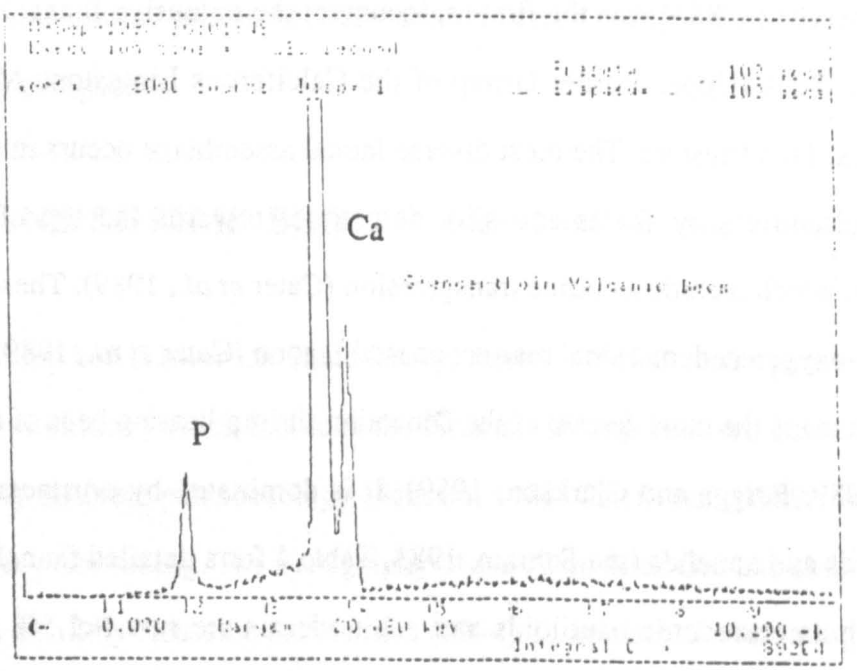
5.2.13 THE GLENCARTHOLM VOLCANIC BEDS (Dinantian, Lower Carboniferous), River Esk, near Langholm, Dumfrieshire, Scotland.

Peach (1882, 1883) was the first to document the extensive fauna of the Glencartholm Volcanic Beds (Upper Border Group of the Calciferous Limestone Measures) along the River Esk, Dumfrieshire. The most diverse faunal assemblage occurs in a 5m thick series of black dolomitic silty shales and silty dolostones towards the top of a fining upwards sequence which records a marine transgression (Cater *et al.*, 1989). These were deposited in a poorly-oxygenated, marginal marine, coastal lagoon (Cater *et al.*, 1989, p8).

The fauna is the most diverse of the Dinantian shrimp-bearing beds of north Britain (Cater *et al.*, 1989; Briggs and Clarkson, 1989). It is dominated by crustaceans, fish, bivalves, gastropods and annelids (see Schram, 1983, Table 1 for a detailed faunal list). Fully marine taxa such as orthoconic nautiloids and echinoderms are rare (<1.5% of the total fauna, Schram, 1983), and some of the associated bivalves are quasi-marine. This, and

sedimentological evidence has led Briggs and Clarkson (1989, p298) to suggest a greater fresh-water influence than formerly suggested by Schram (1981).

The abundance, and taxonomic and lateral distribution of phosphatized soft tissues in the Glencartholm Volcanic Beds is unknown and requires examination. Traquair (1884, Plate 2, fig. 1) figured a specimen (BMNH P5900) of the shark *Ctenacanthus costellatus* in which the skeletal musculature of at least one side of its body was preserved in its entirety, and commented on the occurrence of muscle fibres in the limbs of scorpions from the same locality. EDAX analysis and SEM examination of muscle from BMNH P5900 indicates the soft tissues to be replaced by apatite (see text fig. 5.5 for analysis), and the style of preservation and resolution of detail preserved to be comparable to that of fish from the Romualdo Member (see Section 4.2.2.1). The muscle fibres are replaced by microcrystalline aggregates of apatite, and display clear evidence of transverse banding (fig. 5.18). Most fibres are also partially coated by microbes, which relative to material from the Romualdo Member are considerably more numerous; a single 'mat' of microbes frequently smothering several adjacent fibres (fig. 5.19). These are allied to morphotype 2 microbes of the Romualdo Member (see Section 3.3.2). Most of the microbes are not in contact with the fibres themselves, but are separated by an irregular 'wavy' gap which presumably represents the fibre's sarcolemma.



Text figure 5.5: EDAX elemental analysis of soft tissues from *Ctenacanthus costellatus* (BMNH P5900) from the Glencartholm Volcanic Beds. The elemental peaks are consistent with a hydroxyapatite composition.

Fossilized muscle (presumed to be phosphatized) has also been recorded from another Lower Carboniferous Scottish section. A single specimen (NMS 1983.33.8) of the actinopteran fish - *Mesopoma carricki* sp. nov. (Coates, 1993) - from the Manse Burn Formation at Bearsden (near Glasgow) contains well preserved myotomes (pers. comm. Dr. Coates, University of Cambridge). The Manse Burn Formation (Pendleian, Namurian), much like the Granton and Gullane shrimp beds, and the Glencartholm Volcanic Beds (see Sections 5.2.11, 5.2.12, and 5.2.13 respectively), consists of black, organic-rich, finely laminated marine shales with minor non-marine intercalations. It contains a diverse fauna including actinopterygian fish (Coates, 1988), exceptionally well preserved chondrichthyan fish (Dick *et al.*, 1986), crustaceans (Wood, 1982; Clark, 1990, 1991), conodonts, nautiloids, goniatites, gastropods, bivalves, brachiopods, and crinoids (Wood, 1982). These inhabited an environment subjected to seasonal salinity and oxygen fluctuations (Coates, 1993). It would therefore appear that the Early Carboniferous brackish water environments of northern Britain repeatedly provided the precise conditions required for the phosphatization of soft tissues (see Section 7.4 for a discussion of the most favourable palaeoenvironmental conditions).

5.2.14 THE CLEVELAND SHALE (Late Devonian), Ohio, USA.

The Cleveland Shale (Ohio) is a shallow water, estuarine deposit which forms part of a transgressive sequence (Schwietering, 1977). It outcrops in a north-south band through central Ohio, and consists of a grey/black, organic-rich (>10%, Hoover, 1960) shale containing large (up to 3m diameter) carbonate concretions (see Criss *et al.*, 1988). Both the shale and the concretions contain a scarce fossil assemblage consisting of marine algae, terrestrial plants, conodonts, brachiopods and sharks in that order of abundance (Wells, 1947; Hannibal, 1989).

Dean (1902, 1909) described and figured exceptionally well preserved phosphatized soft tissues from a number of fully articulated sharks (all referable to the genus *Cladoselache*) from the Cleveland Shale. The most exceptional material includes kidneys with nephric tubules, gill filaments, spiral valves of the digestive tract with contents *in situ*, and skeletal muscle in which transverse banding and portions of the sarcolemma are preserved. Dean

(1902, 1909) considered these to have been replaced by inorganically precipitation apatite. Certainly, the exceptional preservation of this material (e.g. see Dean, 1902, figs. 3 and 4) implies replacement by non-spherulitic, microgranular apatite; inorganic microspheres and microbes are in general incapable of replicating tissues to this level of precision (see Section 3.4).

5.2.15 THE ALUM SHALE FORMATION or "ORSTEN" (Upper Cambrian), Sweden and Poland.

The Orsten consists of a phosphatic, pyritic limestone which forms either concretions (10cm-2m in diameter) or flat lenses within the Alum Shale. The limestones are petroliferous and developed beneath a flocculent upper sediment layer (Müller and Walossek, 1986a) in still, marine waters (Müller and Walossek, 1985a, p161). The limestones contain a fauna dominated by small arthropods including trilobites. Conodonts, Chancelloriida, sponge spicules, and brachiopods are locally abundant in lags (Müller and Walossek, 1985a, p161).

Phosphatized soft tissues are restricted to the arthropods (Müller, 1985) of which thousands of specimens have been recovered. Frequently, remarkable details are preserved permitting the biology of the organisms to be accurately reconstructed (e.g. see Müller, 1982a; Müller and Walossek, 1985b; Müller and Walossek, 1986b, 1986c; Müller and Walossek, 1987; Müller and Walossek, 1988). Both coatings and replacements of the soft integument may occur together in the same specimen. Typically, details finer than 1μm are concealed by coatings, and internal organs are usually not fossilized (Müller and Walossek, 1985a, p163). Considerable variation in the quality of preservation occurs within and between outcrops (Müller, 1985 p70), and mineralization is both taxon- and tissue-specific (Müller and Walossek, 1985a, p163; Müller, 1979 p5). Even within individual ostracodes, there is a gradational change in the density of mineralization from the anterior (the most heavily phosphatized end) to the posterior. This probably reflects both the substrate's affinity for nucleating phosphates, and the rate of exoskeleton dissolution versus mineralization (Müller, 1979 p5).

Mineralization of the Orsten fauna was interpreted by Müller (1985) to have been wholly inorganic. However, a number of large spherical bodies replacing the soft tissues of some of

the organisms (see Müller and Walossek, 1985b, figs. 2a and 2c; Müller and Walossek, 1987, Plate 1, fig. 1; Plate 16, fig. 3; Plate 28, fig. 5; Plate 31, fig. 4) closely resemble mineralized microbes from the Romualdo Member (see Section 3.3.2). Allison (1988a p335) and Seilacher (1990 p268) have similarly suggested some of the microfabrics replacing the soft tissues of the Orsten fauna to be microbial in origin. Although there are obvious dangers in interpreting the genesis of microfabrics indirectly from published micrographs, it would appear that microbial mineralization was far more important in preserving the soft tissues of the Orsten fauna than has previously been anticipated.

As with microbes from the Romualdo Member, the spheres replacing soft tissues in the Alum Shale form 'strings' (Müller and Walossek, 1987, Plate 16, fig. 2 [arrowed]), mammillate masses (Müller and Walossek, 1987, Plate 28, fig. 4), occur as isolated individuals (Müller and Walossek, 1987, Plate 20, fig. 3), and form extremely smooth moulds which may be difficult to identify as microbial in origin unless gaps exist between individuals (Müller, 1985, Plate 1, fig. 4; and, Müller and Walossek, 1987, fig. 28a; Plate 19, fig. 5; Plate 25, fig. 5; Plate 31, figs. 3 and 5). Marked differences in the size of the microbes preserving the tissues of organisms from the Alum Shale Formation, suggest at least two microbe morphotypes are present (both allied to morphotype 2 of the Romualdo Member, see Section 3.3.2).

Non-spherulitic inorganic microfabrics also appear to have been involved in the fossilization of the Orsten fauna (see Müller and Walossek, 1987, Plate 8; Plate 32, figs. 5-8), although they are of only secondary importance to microbes. Often, the involvement of non-spherulitic inorganic microfabrics may be inferred by the preservation of structures which are too small to be pseudomorphed by either inorganic microspheres or microbes (e.g. the bristles in Müller and Walossek, 1987, Plate 26, figs. 5, 8, and 9).

There are remarkable similarities in preservational style between the fossilized soft tissues of the Orsten fauna, and those of the ostracodes (Bate, 1972), copepods (Cressey and Boxshall, 1989), and shrimps (Wilby and Martill, 1992) of the Romualdo Member (see Section 4.2.3.2). Phosphatization of taphonomically stable (or recalcitrant, *sensu* Alexander, 1965) tissues (e.g. crustacean cuticles) by a combination of replacement and coatings also

occurs in a number of other deposits (see below). The relative importance of inorganic- and microbially-mediated mechanisms of phosphatization however, varies considerably. Phosphatized soft tissues of ostracodes from the Upper Jurassic (Early Volgian) of the Saratov district (USSR) of the Volga River (Dzik, 1978), the Lower Triassic Sticky Keep Formation of Spitsbergen (Weitschat, 1983a, 1983b), and the Upper Devonian Cephalopod Limestone of the Carnic Alps (Austrian/Italian border)(Müller, 1982b), *appear* to be preserved predominantly by inorganic microfabrics. In contrast, the soft tissues of Crustacea and probable Pentastomida from the Lower Ordovician (Tremadocian) of Sweden (Andres, 1989), ostracodes from the Lower Cambrian of Shropshire (Hinz, 1987), and ammonoids from the Triassic Barents-Øya Formation of Spitsbergen (Weitschat, 1986), have microfabrics *indicative* of both inorganic replacement and microbial infestation.

5.3 CLASSIFICATION OF LAGERSTÄTTEN CONTAINING PHOSPHATIZED SOFT TISSUES

A number of schemes have recently been proposed for the classification of lagerstätten. These have been particularly useful in identifying the common sedimentological-, environmental-, taphonomic-, and temporal-characteristics of these deposits. The most widely accepted scheme is that proposed by Seilacher (1970) in which each deposit is designated to be either a concentration- or conservation lagerstätten. The former refers to deposits containing exceptional concentrations of fossils, whilst the latter describes deposits containing exceptionally well preserved fossil material. Seilacher *et al.* (1985) further divided conservation lagerstätten into three groups (stagnation, obrution, and bacterial sealing) which reflect the chief causative depositional conditions responsible for their exceptional preservation. These define a triangular space into which all such deposits may be mapped.

Allison (1988a and c) recognized the significance of early diagenetic mineral formation to the preservation of most exceptional biotas, and offered an alternative classification scheme (Allison, 1988a). This was based upon fossil mineralogy and mineral paragenesis, and incorporated a variety of depositional parameters including the rate of deposition, organic content, Eh, pH, salinity, and oxygen levels.

More recently, Martill (1990b) and Allison and Briggs (1991b) independently introduced a scheme in which conservation lagerstätten are defined according to the quality/quantity of palaeobiological information preserved in their biota. Each deposit is expressed in terms of its position relative to a number of "taphonomic thresholds" which define the presence or absence of certain organisms and/or tissues.

Allison and Briggs (1991b) also suggested conservation lagerstätten may be classified according to their positions in time and space. This scheme highlighted the dominance of certain preservational environments (or "taphonomic windows" *sensu* Allison and Briggs, 1991b pp42-43) at specific times during the Phanerozoic.

Each of these schemes has played an important role in emphasising the palaeoenvironmental controls on soft tissue fossilization. Unfortunately however, none is capable of expressing the variations in the preservation styles of phosphatized soft tissues that have been outlined in this chapter. This is unfortunate since such a scheme may reveal taxonomic 'trends' in the preservational styles and/or mechanisms of phosphatization of soft tissues.

I present two classification systems which are better equipped to identify taxonomic trends in the preservational styles of phosphatized soft tissues. The data for both schemes are based on the descriptions of phosphatized soft tissues given in Section 5.2. For several deposits, I have had access to only a single specimen containing phosphatized soft tissues and sometimes only one sample from that specimen. My conclusions for some deposits may therefore not be entirely representative of the biota as a whole. The effectiveness of both classification schemes may be tested by the discovery of new deposits containing biotas with phosphatized soft tissues, and by the identification of phosphatized soft tissues in taxa not previously believed to contain such material from any of the deposits discussed in Chapter 5.

1) ACCORDING TO PRESERVATIONAL STYLE:

This system is based on the position of the soft tissues of each Lagerstätte relative to three preservational styles which represent end-members of a continuum of variation (text fig. 5.6).. Since the tissues of different taxa and even individuals of the same taxon within a single deposit may be preserved in a slightly different manner (e.g. see Chapter 4), most

deposits are defined by a small field. Each field defines the relative abundance (a qualitative percentile estimate) of the three preservational styles (see below) in the phosphatized soft tissues of that deposit. The three preservational styles (or end members) are defined as:

1) *Microbial infestation:* here the tissues are preserved solely due to the presence of microbes; the tissues themselves are not mineralized, but rather the invading microbes are. A prolonged period of decay is a prerequisite for this style of preservation so that microbes may gain access to the tissues and become well established. This period of degradation and the large size of many microbes relative to the subcellular structures of most metazoan tissues, prevents extremely fine details from being preserved by microbes (see Section 3.4).

Three styles of microbial infestation may be recognised. These are:

a) *Microbial coating*: the microbes are restricted entirely to the internal and/or external surface of the tissue and therefore preserve only a crude mould of the substrate. This style is exemplified by the phosphatized soft tissues of trigoniids from the Portland Roach (see Section 5.2.4).

b) *Gross microbial replacement*: the microbes pseudomorph the tissue. Gross replacement requires extensive microbial invasion of the tissues and is indicative of a relatively advanced stage of substrate decomposition. This style of preservation is most clearly displayed by the Lower Lias ichthyosaur (DM/Lias/2) described in Section 5.2.9.

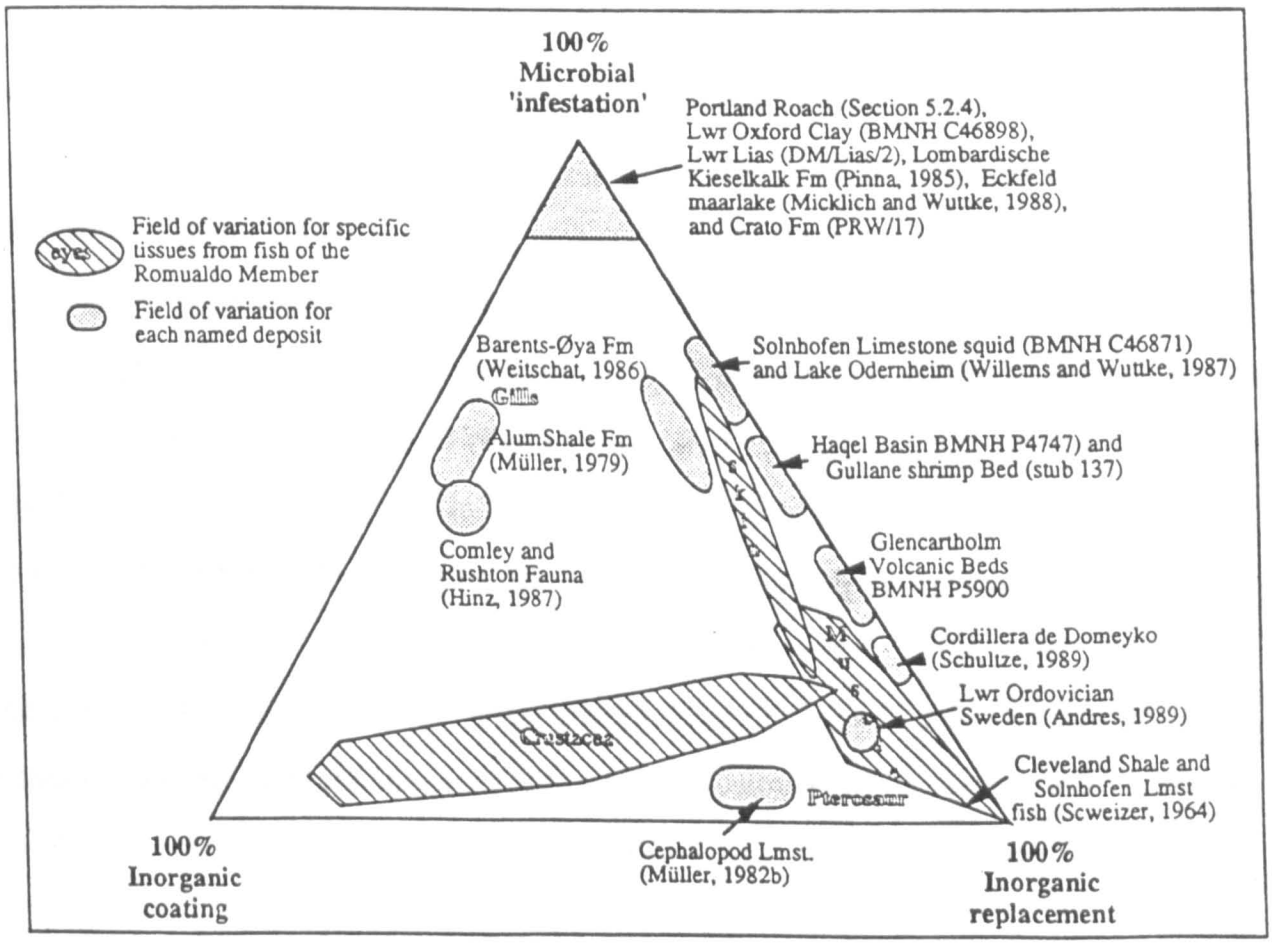
c) *Ultrastructurally controlled microbial replacement:* the distribution of the infesting microbes is controlled by the ultrastructure of the substrate. In the case of striated muscle, this may result in details such as the myofibrils being preserved as in the Portland Roach (see Section 5.2.4).

2) *Inorganic replacement* (or permineralization, *sensu* Allison, 1988a, p334): the soft tissues are replaced either by microgranular apatite (the crystallites of which are either randomly- or preferentially-orientated), or by inorganic microspheres. Although the phosphate ions may have been concentrated microbially (see Sections 1.6.1 and 7.2.1 for a discussion), microbes are not directly involved in the precipitation of the phosphate. This preservational style preserves the greatest resolution of detail and implies phosphates to have been precipitated in the tissues extremely rapidly (relative to decay) from a supersaturated source. Inorganic replacement is exemplified by fish muscle from the Cleveland Shale (see

Section 5.2.14), the Oxfordian of Chile (see Section 5.2.6), and the Romualdo Member (Section 4.2.2.1).

3) *Inorganic coating:* here apatite (either granular or microspherulitic) is deposited directly on an organic substrate as a thin (usually <1μm), more or less continuous blanket. Consequently, details finer than 1μm in diameter are rarely preserved. This style of preservation is most characteristic of recalcitrant tissues such as the exoskeleton of arthropods (e.g. the Orsten fauna, see Section 5.2.15).

Inorganic coatings as defined here are partly synonymous with Allison's (1988a, pp334-335) "mineral coats" whereby " the organisms act as a template for mineral formation". However, Allison (1988a) did not distinguish (as in this study) between those tissues coated by microbes and those coated by inorganic precipitates.



Text figure 5.6: The classification of phosphatized soft tissues according to preservational style. Each Lagestätte is plotted according to the relative importance (a qualitative percentile estimate) of microbial infestations, inorganic coatings, and inorganic replacements in the preservation of soft tissues in its biota. See text for details.

Discussion: Microfabrics and preservational styles are intimately tied to mechanisms of mineralization in this classification scheme. Therefore, with a knowledge of the process(es) responsible for the production of each preservational style (see Chapter 3), the mechanism(s) of mineralization of tissues within any deposit may be assessed based on their position within the triangular fields.

Microbial infestations and inorganic replacements clearly dominate soft tissue phosphatization (text fig. 5.6). In fact, the phosphatized soft tissues of most of the lagerstätten discussed in this chapter display at least some sign of these two processes. Nevertheless, it is clear that taxonomically related groups of organisms are preserved in a similar manner (i.e. they are concentrated into certain regions or broad zones of text figure 5.7) despite having come from different deposits. For example, Coleoidea are restricted to the top third of the triangular field (i.e. microbially infested), whilst fish are largely restricted to the microbial infestation-inorganic replacement gradient.

This scheme clearly has potential for identifying taxon-related trends in preservational style and for emphasising the *prevailing* mechanism(s) of mineralization within each lagerstätte. It is also encouraging to note that even the soft tissues of intensely investigated deposits such the Alum Shale Formation (see Section 5.2.15) display a uniformity in preservational style (i.e. only a small field of variation). This suggests that the position of other less well studied lagerstätten will not be especially dictated by the quantity of material examined. This having been said, the various soft tissues of some organisms even within a single deposit (such as the fish of the Romualdo Member) may be spread over relatively large areas. This probably reflects the relative speed at which microbes may gain access to the different tissues of large, morphologically complex organisms such as fish, and emphasises the tissue-specific nature of soft tissue phosphatization. The scheme's potential as a means of classification is therefore limited.

2) 'MICROTAPHONOMIC' THRESHOLDS:

Martill (1990b), and Allison and Briggs (1991b) independently proposed a scheme in which the quantity of palaeobiological information preserved in fossil biotas is expressed according to their position relative to certain taphonomic thresholds. Each taphonomic

threshold corresponds to the presence or absence of a specific tissue or organism. One may thus define a number of thresholds which require progressively more exceptional conditions to be satisfied (e.g. soft tissues require more exceptional conditions to become fossilized than do 'hard parts'). Since the biotas described in this thesis all contain fossilized soft tissues, differences in the quantity/quality of histological information that they preserve may only be distinguished by 'microtaphonomic' thresholds. Each microtaphonomic threshold corresponds to an increase in the level of ultrastructural information preserved by the fossilized soft tissues. Because they refer to the quantity of information preserved, microtaphonomic thresholds are an indication not only of the extent to which decay had advanced prior to mineralization, but also of the style (or mechanism) of preservation.

Five microtaphonomic thresholds are recognised:

1) *Structureless:* soft tissues are preserved, but due to sedimentary compaction, tissue collapse (see Zangerl, 1971), and/or rapid microbial degradation (relative to mineralization), individual organs are not discernible. The phosphatized material is entirely structureless.

2) *Whole organs:* Individual organs are preserved but they display no ultrastructural detail.

3) *Cellular:* cellular details are preserved. In the case of muscle, only the gross outline of the fibres is discernible.

4) *Subcellular:* subcellular structures are preserved. In the case of striated muscle, the sarcomeres, organelles, sarcolemma, connective fibrils, capillaries, and the system of T-tubules can be distinguished.

5) *Macromolecular:* individual molecules are replaced by apatite. This can only be demonstrated with high resolution TEM and micro-electron-diffraction investigations of tissues containing macromolecules with a preferred orientation (e.g. the collagen fibres of dermis or the actinomyosin complex of muscle).

Each deposit may be displayed as a line (or series of lines, see below) whose shading reflects the process(es) by which its soft tissues were phosphatized (table 5.1). The processes of phosphatization (as defined for the previous scheme, see above) in order of decreasing precision with which they replicate the soft tissues are: 1) inorganic replacement,

2) inorganic coating, and 3) microbial infestation. "Uncertain" refers to those tissues for which it has not been possible to determine the style of preservation. Each group of organisms within an individual deposit is displayed as a single line (if their preservation is distinct from that of other groups). Composite shading demonstrates the range of preservational styles which occur in different tissues within a single group of organisms. The position of the various preservational styles on any composite line corresponds to the resolution of detail preserved by that particular preservational style.

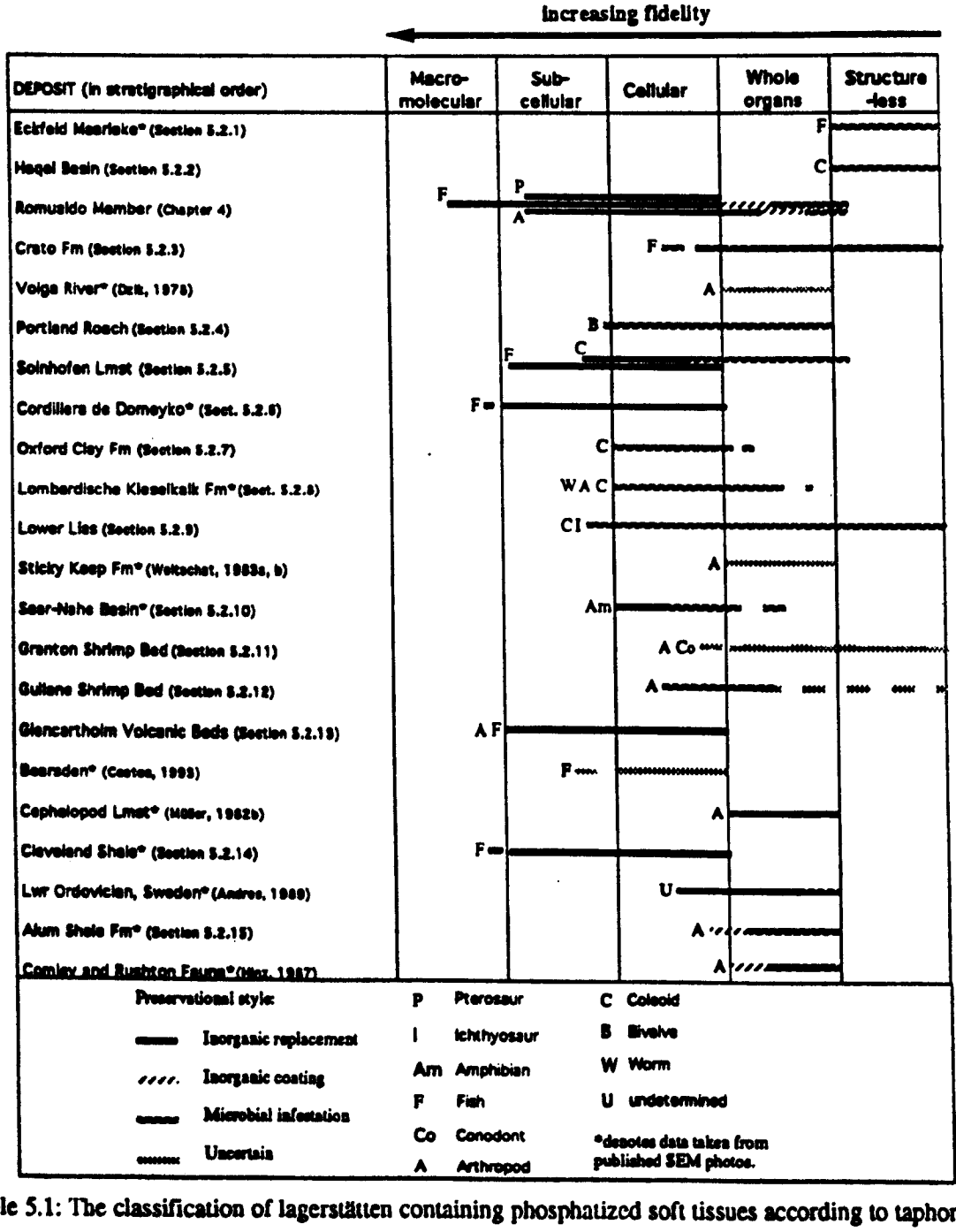


Table 5.1: The classification of lagerstätten containing phosphatized soft tissues according to taphonomic thresholds. Each deposit is displayed as a line (or series of lines) whose shading reflects the process(es) by which its soft tissues were phosphatized ("uncertain" refers to those tissues whose preservational style requires examination). See text for details.

Discussion: This classification system accentuates variations in preservational style both within individual organisms, and between different groups of organisms. It permits one to predict the style of preservation of soft tissues which have not yet been examined. For example, from the position of the Manse Burn Formation in text figure 5.9, one may predict the muscle fibres of *Mesopoma carricki* (see Section 5.2.13) to be inorganically replaced.

This scheme also provides useful information regarding the resolution of detail effected by each preservational style. It is clear that inorganically replaced soft tissues display the greatest resolution of detail (commonly to subcellular level); inorganic coatings preserve only whole organs (e.g. the appendages of arthropods); and that microbial infestations are generally incapable of preserving more than cellular details (e.g the outline of muscle fibres). Microtaphonomic thresholds also demonstrate arthropods to be characterized by 'whole organ' preservation (i.e. the appendages are well preserved but internal soft tissues are exceedingly rare), and the most exceptionally well preserved soft tissues to occur in fish. Unfortunately however, without considerably complicating the scheme, microtaphonomic thresholds are incapable of representing the relative importance of preservational styles in each deposit, organism, or tissue.

5.4 CONCLUSIONS

1) The phosphatized soft tissues of several lagerstätten including the Portland Roach, the Glencartholm Volcanic Beds, and the Cleveland Shale have not received attention since before the widespread availability of SEM's. These, and the soft tissues preserved in a number of newly discovered localities (e.g. the Manse Burn Formation and a Cambrian Burgess Shale-type deposit in Greenland [pers. comm. Graham Budd, University of Cambridge]) require examination.

2) Phosphatized soft tissues are preserved in sediments at least as ancient as the Lower Cambrian (Hinz, 1987), and occur in marine- (e.g. Oxford Clay Formation, see Section 5.2.7), brackish- (e.g. Granton shrimp bed, see Section 5.2.11), and freshwater- (e.g. Eckfeld Maarlake, see Section 5.2.1) sediments. They are therefore both temporally and environmentally widespread.

3) Phosphatized soft tissues from different deposits may be preserved by strikingly similar microfabrics and preservational styles. All material examined in this study can be expressed in terms of one or more of the three preservational styles - phosphatized microbial infestations, inorganic phosphatic coatings, and inorganic phosphatic replacements. This suggests that one or more of these three processes are involved in the phosphatization of all soft tissues.

4) Microbial infestations are by far the most common preservational style/mechanism of phosphatization.

5) Each preservational style preserves a predictable level of detail. Microbial infestations rarely preserve more than cellular details; inorganic coatings most frequently preserve only whole organs; and, inorganic replacements most characteristically preserve sub-cellular details (but may replace macromolecules).

6) In general, comparable tissues within related taxa from different deposits are similarly preserved. The preservation of soft tissues in arthropods is dominated by microbial infestations and inorganic coatings; coleoids by microbial infestations; and fish by inorganic replacements and microbial infestations. The soft tissues of different groups of organisms therefore display a characteristic level of detail. Arthropods are characterised by the preservation of whole organs, whilst subcellular details are the norm in fish.

CHAPTER 6
PHOSPHATIZED SOFT TISSUES: THE TIMING OF MINERALIZATION

6.1 INTRODUCTION

A realistic estimate of the timing of phosphatization of soft tissues is essential if one is to establish which particular tissues, biomolecules and chemical species were intact and therefore available to act as sites for the nucleation of apatite. Only with such a knowledge can one assess the quantity of information obscured, destroyed and/or not preserved by the mineralizing process. Previous investigations have proposed mineralization to have been pre-mortem (Schultze, 1989), the cause of death (Bate, 1972, Müller, 1985), 1-2 hours postmortem (Martill and Harper, 1990), and 2^{+} weeks postmortem (Briggs and Kear, 1993a). These conflicting views have led to the development of a number of very different models of soft tissue phosphatization (see Section 1.6.2).

This chapter provides a semi-quantitative estimate of the timing of phosphatization of soft tissues in the Romualdo Member, and an assessment of the salinity of the bottom waters of the Romualdo Lagoon. These are based on the timing of appearance in experimentally decayed soft tissues of taphonomic features comparable to those preserved in the fossil soft tissues. I also comment on the relative timing of mineralization in other deposits.

6.2 MICROTAPHONOMY (the taphonomy of cellular and sub-cellular structures).

Failure to recognise evidence of decay in the phosphatized soft tissues of many deposits has prompted most workers to propose mineralization as having been either a pre-mortem or *very* early postmortem event (see Section 1.6.3). In fact, taphonomic structures (or taphostructures) may be identified in the majority of phosphatized soft tissues. For example, in fish from the Romualdo Member, secondary gill lamellae are usually in a state of collapse (see Martill and Harper, 1990), and many originally globose cells such as those of the stomach wall display considerable evidence of shrinkage (fig. 6.1). These taphostructures, together with the frequency with which phosphatized soft tissues are infested by microbes

(see Section 5.3), provide irrefutable evidence of phosphatization having post-dated a period of decomposition.

It is unfortunate that the sequence, timing, and processes of decay of most organisms in the aquatic realm, particularly at the cellular level, are not well understood (although see Briggs and Kear, 1993b on polychaetes; Allison, 1988c, 1990, and Briggs and Kear, in review on crustaceans; Martill and Harper, 1990 on fish; and Gordon *et al.*, 1988 on humans). Such data are essential to any estimate of the timing of mineralization of phosphatized soft tissues. Forensic (e.g. see Gordon *et al.*, 1988) and food science investigations provide obvious potential sources of data but it is unlikely that the decomposition of human cadavers (usually anhydrous) and the spoilage of prepared and frozen foods can be extrapolated directly to palaeontological situations. I therefore present a pilot microtaphonomic study of the progressive stages of decay of striated fish muscle:

6.2.1 THE DECAY OF STRIATED FISH MUSCLE

Although taphostructures may be identified in the majority of soft tissues from the Romualdo Member, for a number of reasons striated fish muscle represents the most practical reference tissue on which to base an estimate of the timing of mineralization. In particular:

1) Striated muscle is the most commonly preserved soft tissue in the Romualdo Member and is therefore available in abundance.

2) Although the environmental controls on microbial respiration (see Allison and Briggs, 1991a for a summary), and the chemical factors dictating the rates of decay of organic carbon (Alexander, 1965; Allison, 1988a, fig. 6) particularly when dispersed throughout sediments (see Alexander, 1973; Berner, 1981; Emerson and Hedges, 1988) are well established, the effects of local microbial populations on individual tissues are not easily quantified. Most of the skeletal muscle preserved in fish from the Romualdo Member is devoid of microbes (see Section 4.2.2.1). Therefore, assuming the distribution of microbial enzymes to be restricted largely to their own immediate vicinity (an assumption *perhaps* supported by the development of 'microbial pits' in some fossil soft tissues, see Section 3.3.1), one can assume the decay of muscle in the fish of the Romualdo Member to have

been dictated *largely* by osmotic fluctuations and the action of the tissue's indigenous enzymes (i.e. autolysis). This diminishes the as yet largely unquantified variables associated with the microbial degradation of chemically heterogeneous organic material (e.g. soft tissues). It does not however, completely eradicate the potential influence that microbial enzymes may have had on the decay of these soft tissues. Indeed, it is difficult to envisage microbes as not having influenced the process of decomposition to some extent, particularly during the most advanced stages of decay. Microbes proliferate extremely rapidly and it is easy to demonstrate that those infesting carcasses do affect the chemical microenvironment of decaying tissues (e.g. see Berner, 1968a; Allison, 1988c; Briggs and Kear, 1993a; text fig. 8.5). However, since there is no direct evidence in most of the fossil fish muscle from the Romualdo Member of microbes, their influence was probably only minimal. This assumption is strongly supported by the results of control experiments in which the size of the microbial populations of the water surrounding decaying fish were controlled. For at least the first 60 hours postmortem (at 21^0C and 3.5% salinity), the rates of decay and appearance of muscle (see Section 6.2.1.2) from fish decomposed in sterile marine water (refluxed for 1 hour and sterilised at the beginning of the experiment with 5ml of Miltons sterilising fluid) was virtually identical to that of similar fish decomposed in water seeded with microbes (as described in Section 6.2.1.1.).

3) The structure (see text fig. 4.2) and chemistry of striated muscle is extremely well understood (see Alberts *et al.*, 1989, pp613-624) and therefore, the progressive stages of its decay may be easily followed. The abundance and variety of subcellular structures in muscle (see text fig. 4.2) also permit a number of different taphostructures to be cross-referenced with one another (Wilby, 1992).

4) The choice of a striated muscle as opposed to gills (see Martill and Harper, 1990) permits the relative potential of these two tissues as a 'decay stop-clock' to be gauged.

5) Although the soft tissues of fish are not preserved in all of the deposits discussed in Chapter 5, muscle is preserved in at least one organism in many of them. The state of preservation of striated muscle has thus received the greatest attention of any tissue in the literature (e.g. see Dean, 1909; Pinna, 1985; Willems and Wuttke, 1987; Allison, 1988d; Schultze, 1989; Martill, 1990a; Mehl, 1990).

6.2.1.1 EXPERIMENTAL PROCEDURES

Live Atlantic Cod (*Gadus morhua*) were killed by a single blow to the head after overnight travel, and placed in 4 litre plastic bowls containing 2.5 litres of artificial sea water (3.5% salinity). The water had been made up at least two weeks in advance and inoculated with bacteria from a water/slurry mixture of a previous taphonomic experiment. Each bowl was covered with a plastic sheet (not completely air-tight) and maintained in the dark at 20^0C. The original salinity was maintained by monitoring water levels and making adjustments with distilled water when necessary.

At the time of death, and at the following intervals postmortem (given in minutes or hours/minutes) - 10mins, 45mins, 1/40, 2/25, 3/0, 3/40, 4/25, 5/0, 6/0, 22/0, 24/30, 27/0, 30/0, 46/0, 52/30, 58/30, 73/0 - the carcasses were removed from the decay vessels and the outer surface of a $3cm^3$ area dried by applying tissue paper. From these sites, at a depth of approximately 5mm, two pieces of skeletal muscle (each approximately $3mm^3$) were dissected and immediately processed for TEM examination according to the schedule given in appendix 3iiia. A depth of 5mm was chosen since muscle at this depth in fish from the Romualdo Member is devoid of microbes. To reduce the potential influence of age and sex related variables, each experiment consisted of at least two fish from which samples were randomly selected at each time interval. Two repeat experiments were also simultaneously performed as standards. To be consistent with the average size of the specimens of *Notelops* and *Rhacolepis* from the Romualdo Member from which phosphatized soft tissues has been recovered, all cod used were approximately 30cm in length.

6.2.1.2 RESULTS, INTERPRETATION AND COMPARISON WITH FOSSIL MUSCLE

Decay in all three parallel-run experiments followed identical paths. The successive stages in the decomposition of striated muscle and an interpretation of the processes involved are given below independently for each muscle component. Taphostructures produced in the experiments are compared with structures preserved in fossil muscle from the Romualdo Member and other Largerstätten.

NUCLEI: Striated muscle cell nuclei are easily identified in TEM (fig. 6.2 and text fig. 4.4). They appear as 'sausage-shaped' or spherical bodies (depending on the plane of the section) with a diameter of approximately 1.5μm. The nuclear envelope (which consists of two closely spaced membranes) is taut in living nuclei, and the chromatin is concentrated along the inside of the inner membrane. Divergence from this morphology increases with decay. Four particularly characteristic stages may be recognised:

1) The nuclear envelope becomes progressively less taut and the organelle's globose morphology becomes more irregular in outline. The internal characteristics remain largely unchanged (fig. 6.3).

2) Severe divergences from the original outline develop (essentially deflation) accompanied by the separation of the envelope's two membranes, and a more even dispersal of the chromatin (fig. 6.4).

3) Degradation of the chromatin and damage to the nuclear envelope continues until 'empty spaces' develop in the nuclear lumen (fig. 6.5).

4) The envelope is breached and the nuclear contents are dispersed (fig. 6.6).

MITOCHONDRIA: These subspherical organelles consist of an outer and inner membrane, the latter being folded inwards into a complex series of cristae (see fig. 6.7 and text fig. 4.6). With the advance of decay, the outer membrane becomes progressively more irregular in outline, and the number, clarity, and size of cristae decreases (fig. 6.8). Prior to complete disintegration, the outer membrane separates from the inner one and the organelles deflate (fig. 6.9).

Interpretation of events in the decay of organelles: It is useful to consider the degradation of nuclei and mitochondria together, so that the decomposition of these two similarly sized, but structurally very different membrane-bound organelles may be compared.

The morphological changes which occur in membrane-bound bodies (such as organelles) immediately after death have been documented in detail (see Trump *et al.*, 1984). These

result predominantly from exchanges in solutes between the organelles and the external body fluids as a new osmotic balance is established. At the point of death, the electrochemical potential across each organelle's membrane(s) (which is maintained in life by ATPase transmembrane ion pumps, see Alberts *et al.*, 1989, pp300-323) gradually diminishes as each ion diffuses back down its electrochemical gradient. Due to the presence internally of highly charged macromolecules, the concentration of solutes within the organelles at this new state of rest is greater than that of the extracellular fluids (see Alberts *et al.*, 1989, p308 for explanation). This encourages a net influx of water into the organelles and causes swelling.

Although these processes undoubtedly play a role in the initial decomposition of the organelles of striated fish muscle, the tendency of both nuclei and mitochondria in the experiments described above to 'deflate' rather than become bloated, suggests that it was only minor. Instead, it appears that the salinity of the external environment had a more dominant role in producing the observed morphological changes. The greater salinity of sea water (3.5%) compared with that of physiological fluids (0.9%) (Moyle and Cech, 1982, p75, 79), appears to have caused a net diffusion of ions into, and efflux of water out of the carcasses, thus causing the nuclei and mitochondria to 'shrink' (figs. 6.5 and 6.9 respectively). The rate at which this occurs in nuclei is considerably quicker than in mitochondria, because the envelope of the former is penetrated by large aqueous channels (nuclear pores)(see text fig. 4.4) which permit all small molecules (up to 5000 daltons, Alberts *et al.*, 1989, p423) to diffuse between the lumen and external space. In contrast, although the outer membrane of mitochondria is permeable to all molecules smaller than 10000 daltons (Alberts *et al.*, 1989, p343), the inner membrane is relatively impermeable, thereby delaying osmotic equilibration.

Osmotic equilibration is also likely to be the cause of the irregular gap which frequently develops between the inner and outer membranes of both nuclei and mitochondria (figs. 6.9 and 6.6 respectively). In nuclei, minor differences in the ionic concentration of the perinuclear space relative to that of the surrounding fluids may be the cause. In mitochondria, separation of the two membranes is more likely to be the result of differences

in the permeability of the inner and outer membranes, and thus rates at which they respond to the osmotic changes described above.

Interestingly, Briggs and Kear (1993b) have demonstrated experimentally that a net uptake of water takes place at more advanced stages in the decomposition of soft tissues. This, they (Briggs and Kear, 1993b, pp127-128) proposed to be related to the escape of putrefaction gases from the carcasses.

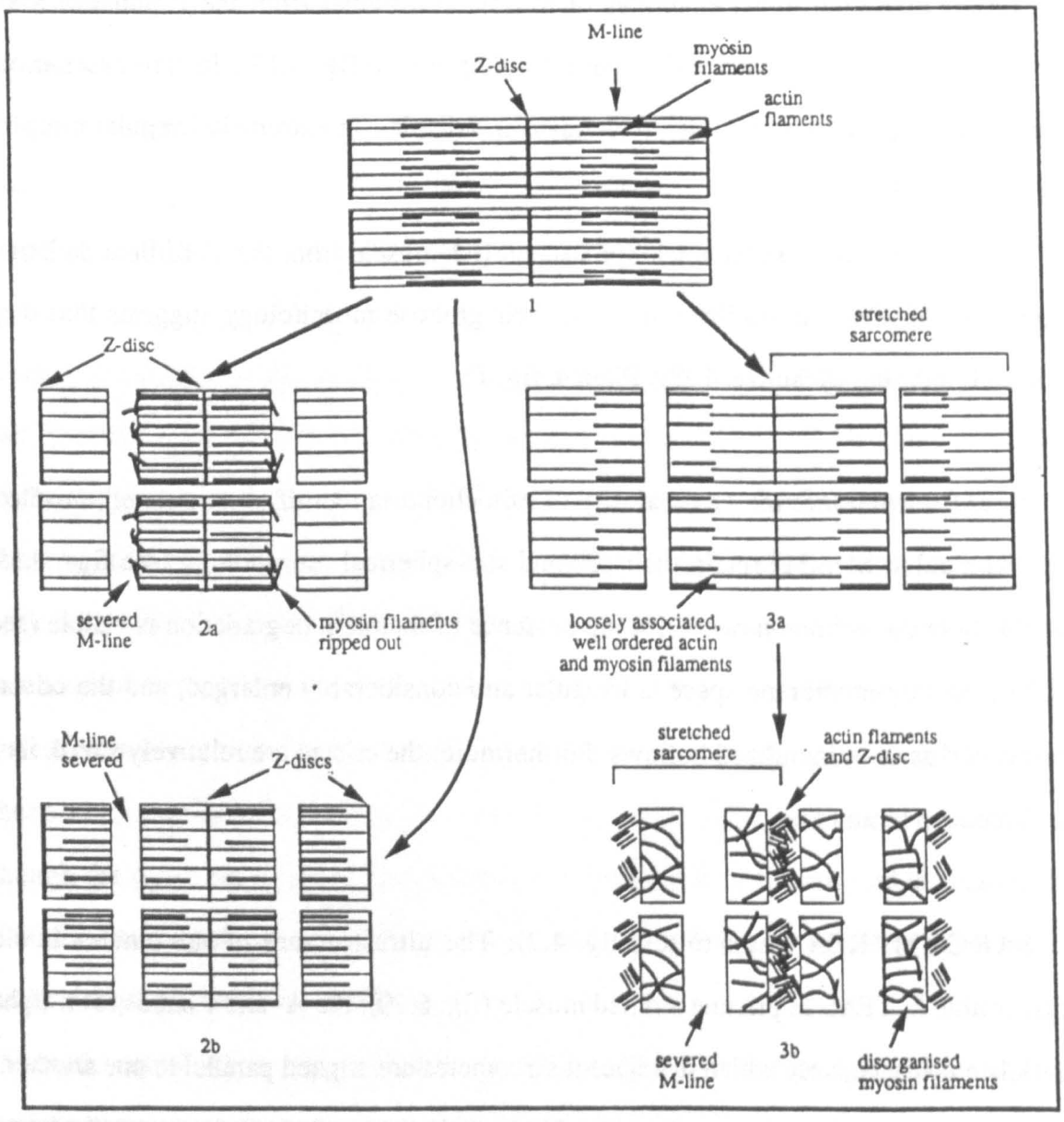
Fossil nuclei: In most examples of muscle cell nuclei from the Romualdo Member, evidence of decay is restricted to partial collapse (see fig. 4.17). In rare cases however, some nuclei are almost completely deflated and display an extremely irregular morphology (see fig. 4.13).

Evidence of decay in the nuclei of striated fish muscle from the Cordillera de Domeyko region of N. Chile is similarly limited; their globose morphology suggests that they are virtually pristine (Schultze, 1989, Plate 4, fig. 1).

Fossil mitochondria: The majority of mitochondria identified in striated muscle from the Romualdo Member retain their original sub-spherical morphology (see figs. 4.19 and 4.21). In one specimen however, some evidence of incipient degradation is visible (see fig. 4.20); the intermembrane space is irregular and considerably enlarged, and the cristae are preserved as un-mineralized hollows. Furthermore, the cristae are relatively small, isolated and reduced in numbers.

SARCOMERES (refer to text fig. 4.2): The ultrastructure of sarcomeres is clearly discernible in TEM. In pristine striated muscle (fig. 6.10), the A- and I-bands form light and dark bands respectively which in adjacent sarcomeres are aligned parallel to one another. The ordered arrangement of actin and myosin filaments is clear and at regular intervals along each myofibril (every ≈0.6μm), the sarcomeres are 'dissected' by thin, dark, continuous lines - the Z-discs and M-lines. This ordered and characteristic structure however, is corrupted even after a short period of decay.

Soon after death (a matter of hours), striated fish muscle undergoes immense contraction (rigor mortis). This strain is alleviated by the rupture of each sarcomere along its M-line (fig. 6.11). The rather uneven distribution of thin dark bands on either side of a few of these dislocations, suggests the tension in *some* myofibrils to be relieved by physically ripping the myosin filaments away from the actin filaments of the other half of the sarcomere (text fig. 6.1:2a). In the majority of sarcomeres however, the release of stress is accomplished by severing the myosin filaments along their bare zones, or M-lines (text fig. 6.1:2b).



Text figure 6.1: Three alternative mechanisms of producing taphonomic banding in striated muscle. 1) four pristine striated muscle sarcomeres (myofibrils running E-W). Seee text for details.

At this point, the tissue's morphology may evolve in one of two ways:

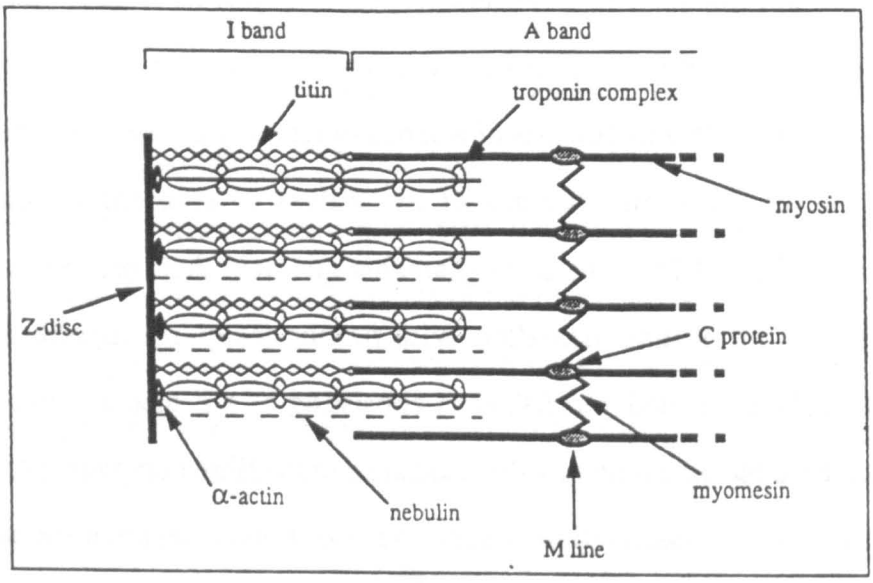
i) In those fibres in which the actinomyosin bonds are not severed, further contraction results in any remaining myosin filaments bridging the enlarged M-lines to be broken. This

results in the development of transverse ruptures across each sarcomere which become progressively more pronounced and considerably less regular with the advance of decay (fig. 6.12). The muscle then consists of a series of laterally connected Z-discs each forming a 'wall' to which the actin filaments of two halves of adjacent sarcomeres are firmly anchored (text fig. 6.1:2b). The actin filaments maintain their ordered arrangement for a considerable period of time thereafter. With further decay, the myofibrils degrade into a series of loosely associated 'blocks', each consisting of a Z-disc and two half sarcomeres (fig. 6.13). These blocks eventually disassociate and the fibre completely disintegrates.

ii) Alternatively, if contraction is such that the 'bonds' between the actin and myosin filaments are irreparably damaged, the sarcomeres adopt a 'stretched' morphology (fig. 6.14; text fig. 6.1:3a). That is, the actin and myosin filaments although still well ordered, are only loosely associated with one another, contact being limited to their terminal ends. Continued tension results in further 'slippage' between the two sets of filaments until the actin filaments are pulled free (still attached to the Z-disc) and lie flaccid in clusters between blocks of completely disorganised myosin filaments (fig. 6.15; text fig. 6.1:3b). At this point, the muscle fibres display complex banding patterns composed of the ruptured M-lines, actin filaments, and structureless blocks of myosin (fig. 6.16). With further decay, complete disintegration ensues.

In both sequences, the loss of fibre integrity and decay of the myosepta causes the fibres to separate from one another, both normal to their length, and across the myosepta.

Interpretation of events: Immediately following death, sarcomeres relax (see fig. 6.10) due to an unlinking of the actin and myosin filaments (Pitcher and Hart, 1982), and then experience massive simultaneous contraction (rigor mortis). Rigor mortis is stimulated by the leakage of Ca^{2+} from the T-tubules surrounding each sarcomere and results in the rupture of many sarcomeres along their M-lines (see fig. 6.11). Ruptures develop most readily along the M-lines because these are inherently weak zones where identically charged myosin molecules abut and are stabilized by a complex of accessory proteins (predominantly myomesin and C protein, Alberts *et al.*, 1989, Table 11-1; refer to text fig. 6.2).



Text figure 6.2: A schematic half sarcomere (running E-W) illustrating the position of the accessory proteins.

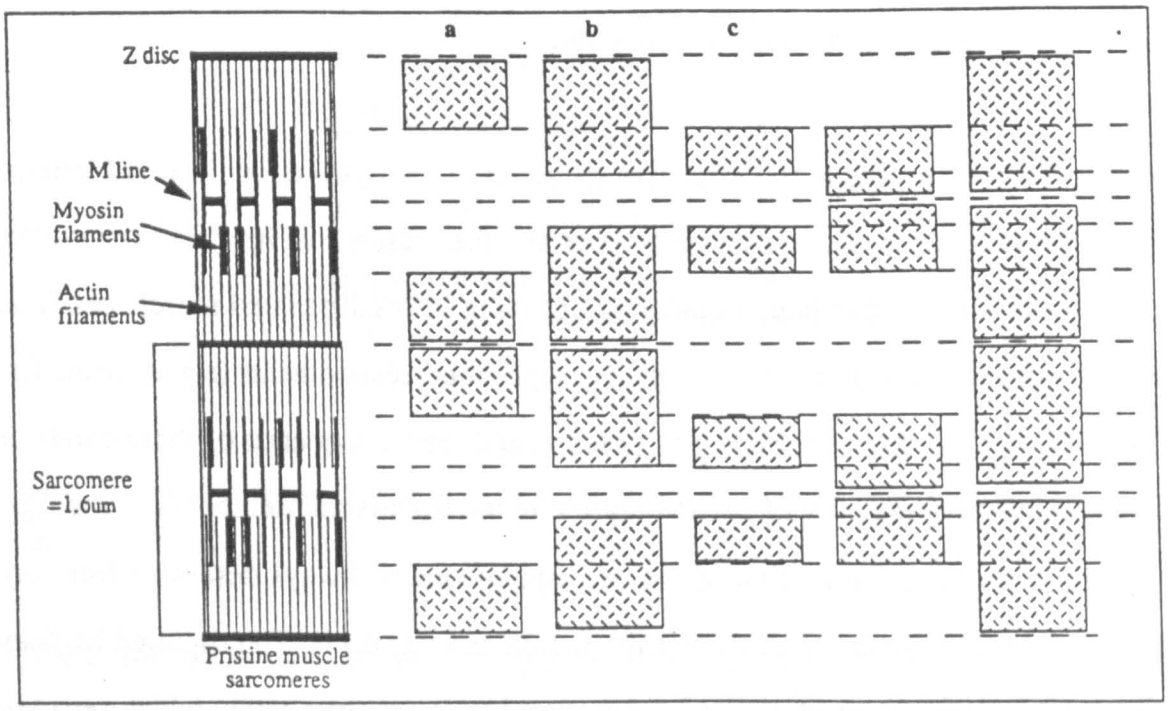
The influx of Ca^{2+} also activates indigenous lysosomal enzymes which eventually resolve the rigor mortis by disrupting the actinomyosin bonds and hydrolyzing some of the muscle's proteins (Pitcher and Hart, 1982). This encourages the ruptures produced by rigor mortis to further widen (fig. 6.12). In some fibres (i.e. those in which decay results in the pattern of banding schematized in text fig. 6.1:2b), despite a considerable loss of structural integrity, the ordered arrangement of actin and myosin filaments relative to one another is maintained (see fig. 6.13) throughout decomposition due to the elasticity of the two accessory proteins - titin and nebulin (see text fig. 6.2). The arrangement of the actin and myosin filaments is further stabilized by the curious disposition of both types of filaments to spontaneously aggregate *in vitro* from their respective constituent molecules at physiological salinities (see Alberts *et al.*, 1989, pp 616-617).

In contrast, in cases where contraction has been especially pronounced resulting in the complete disassociation of the actin and myosin filaments (i.e. those in which decay results in the pattern of banding schematized in text fig. 6.1: 3a), the titin molecules are severed and the half myosin filaments become disorganised (text fig. 6.1: 3b). Despite this, the mutual spacing of the actin filaments is maintained (fig. 6.15) by by nebulin and the presence of the stiffening accessory protein - tropomyosin (refer to text fig. 6.2).

As decomposition proceeds beyond the state described thus far, lysosomal enzymes continue to hydrolyze the fibre's proteins. This results in further disorganisation of the actinomyosin complex and the collapse of the fibres.

Fossil sarcomeres: The most distinctive evidence of autolytic decay in fossil striated muscle is the abundance of ruptured sarcomeres and the presence of irregular, transverse, taphonomically induced banding. Taphonomic banding in fossil striated muscle can look remarkably similar to that produced in actualistic taphonomic experiments (e.g. compare fig. 6.17 with fig. 6.13). Based on the width, spacing, and repeat sequence of these bands, it would appear that the majority of striated muscle in the Romualdo Member (e.g. see figs. 4.7, 4.8, 4.17) decayed via the first of the two taphonomic pathways described from my experiments (i.e. that summarised by text fig. 6.1:2b and fig. 6.11). The ruptured M-lines are preserved as unmineralized, irregular transverse bands (<1-2μm wide) which separate bands of mineralized muscle corresponding to the severed sarcomeres. Each mineralized band is itself divided into two by a thin (<100nm) unmineralized zone (see fig. 4.7) which represents the former position of the Z-discs. In some fossil striated muscle, the ruptured M-lines are extremely wide (>10μm) and irregular (fig. 6.18). This is indicative of a very advanced state of decay.

Examples of the alternative sequence of decay observed in the taphonomic experiments (i.e. that summarised by text fig. 6.1:3b) are rare (but not unknown) in fossil striated fish muscle. In the Romualdo Member, such fibres are characterized by a simple repetition of relatively wide (≈1.2μm) unmineralized bands, and thin (≈0.5μm) mineralized bands (fig. 6.19). Each unmineralized band is of a constant width along its entire length, and is of a similar dimension to other unmineralized bands of the same fibre. This width is comparable to the distance recorded in the taphonomic experiments between 'clusters' of flaccid actin filaments (see fig. 6.15), and therefore probably corresponds to the disorganised blocks of myosin filaments. According to this assumption, the mineralized bands of these fibres must then represent the intervening clusters of flaccid actin filaments. This is supported by the slightly irregular outline of these mineralized bands, and their comparable width to the clusters of actin filaments in the taphonomic experiments.



Text figure 6.3: Differential mineralization of two pristine striated muscle sarcomeres (running N-S). Selective mineralization of certain regions of the actinomyosin complex of the sarcomeres can produce distinctive banding patterns with large gaps between each mineralized band (shading indicates mineralized areas). The spacing of these are not however the same as in fossil phosphatized muscle. a, b, c indicate the selective mineralization suggested by Schultze (1989). See text for details.

Taphonomically induced banding also occurs in striated fish muscle from the Glencartholm Volcanic Beds (see fig. 5.18); the Solnhofen Limestone (Schweizer, 1964, Plate 10, figs. 6-8), the Cordillera de Domeyko (Schultze, 1989, Plate 3, figs. 5 and 6; Plate 4, figs. 1 and 2), and the Cleveland Shale (Dean, 1909, fig. 36), and has been reported in muscle phosphatized *in vitro* (Briggs and Kear, 1993a, fig. 2a). The genesis of this banding however, has often been misinterpreted:

Dean (1909) described banding in Devonian shark muscle from the Cleveland Shale (USA), and noted it to be $^1/_3$ less numerous than in Recent fish muscle. This he (Dean, 1909) proposed, reflected the primitive construction of the fossil muscle. Such a conclusion however is unnecessary if one takes into account the production of ruptures in decomposing muscle, and the corresponding increase in the width of the M-lines. The lower density of banding observed by Dean (1909) may then be interpreted as as being the result of the same number of bands being preserved in a taphonomically elongated fibre.

Schultze (1989) suggested banding in phosphatized muscle from the Jurassic of Chile to have been produced in *live* muscle from the mineralization of: a) only the portions of the actin filaments which are not linked to myosin filaments, b) the entire actin filaments, or, c) only the portion of the actin filaments which are linked to myosin filaments (respectively a, b, and c in text fig. 6.3). However, although differential mineralization of various biomolecules in pristine striated muscle may produce distinctive banding patterns with large gaps between each mineralized band (see text fig. 6.3), it is incapable of producing unmineralized bands with variable widths which are so characteristic of the fossil muscle fibres. Such banding may only be produced by the massive simultaneous contraction of every sarcomere during rigor mortis.

SARCOLEMA AND CONNECTIVE TISSUES: Sarcolemma disintegration is synchronous with that of the fibres. Relatively rapidly postmortem, the sarcolemmas lift free from the underlying myofibrils and break-up into small sections prior to complete disintegration. Despite the apparent fragility of these membranes, the distinctive banding of their constituent collagen filaments (see Miller, 1984) remains visible in TEM almost to the point at which the entire fibre disintegrates.

In contrast, the connective tissues accompanying each muscle fibre (e.g. the Z-discs, the collagenous filaments extending from the terminal ends of each fibre, and the myosepta) display little evidence of decay until the fibres themselves are verging on complete collapse. Similarly, the accessory proteins of striated muscle are taphonomically stable (or recalcitrant), a fact attested by the period over which the actin and myosin filaments retain their original ordering (see above).

Interpretation of events: The coincidental disintegration of the sarcolemmas and muscle fibres suggests damage to the membrane to be the result of stresses imposed on it by rigor mortis in the myofibrils. This is supported by the retention of banding in the collagen filaments comprising the sarcolemmas which implies the membrane's fragmentation not to have been enzymatically controlled.

The structural stability of the connective tissues and accessory proteins of striated muscle suggests that relative to actin and myosin, these molecules are chemically recalcitrant.

Fossil sarcolemmas and connective tissues: Although only rarely preserved, the sarcolemmas of striated fish muscle in the Romualdo Member display indisputable evidence of incipient decay. Most are merely contorted and have separated from the myofibrils (see fig. 4.15), but some have been severely ruptured by tension stresses caused by the collapsing sarcomeres (fig. 6.20).

The sarcolemmas of fish muscle from other deposits similarly display evidence of decay. In the Glencartholm Volcanic Beds (see Section 5.2.13), sarcolemmas are frequently preserved as a thin, 'wavy' unmineralized gap which prevent the underlying myofibrils from being infested by microbes (see fig. 5.19). In the Cleveland Shale (see Section 5.2.14), only short sections of sarcolemma are preserved (Dean, 1902, fig. 3) implying disintegration to have been at a relatively advanced state prior to mineralization.

In contrast, the connective fibrils protruding from the terminal ends of muscle fibres in fish from the Romualdo Member are extremely well preserved. Frequently, these display a regular periodicity of thickenings which may correspond to the original banding of the collagen filaments (see fig. 4.14). Connective fibrils have not been observed or recorded from any of the deposits discussed in Chapter 5.

Phosphatized Z-discs have not been recorded from the muscle of any deposit, but their presence at the time of mineralization (as predicted from the taphonomic experiments) may be inferred from the occurrence of thin (<100nm), unmineralized 'slits' in muscle from the Romualdo Member (see fig. 4.7), and the Cordillera de Domeyko (Schultze, 1989, Plate 3, fig. 6).

Myosepta are only very rarely preserved in striated fish muscle from the Romualdo Member, and are always pseudomorphed by dense populations of microbes. Myosepta have not been recorded from any other deposits containing phosphatized soft tissues.

T-TUBULES AND SARCOPLASMIC RETICULUM: The complex network of T-tubules and sarcoplasmic reticulum in striated muscle experiences massive distortion

during rigor mortis. Much of the network however, recovers its original morphology when the sarcomeres are ruptured and the myofibrils relax. Although displaying signs of 'shrinkage', some of the T-tubules and a little of the sarcoplasmic reticulum remains virtually intact up until the complete disintegration of the muscle fibres (see fig. 6.13).

Interpretation of events: The extreme levels of distortion displayed by the T-tubules and sarcoplasmic reticulum during rigor mortis result from their intimate association with the contracting sarcomeres. Shrinkage of the T-tubules much later on in decay, probably result from a reduction in pressure of the fishes' body fluids following the development of lesions in their body walls.

Fossil T-tubules and sarcoplasmic reticulum: Although only rarely preserved, evidence of decay is not obvious in the T-tubules and sarcoplasmic reticulum of striated fish muscle from the Romualdo Member. Both structures retain their original spherical cross-section profiles (see figs. 4.18, 4.24).

MICROBIAL INFESTATION: At depths of 5mm beneath the dermis, the role of bacteria in the initial stages of skeletal muscle decay (prior to the complete disorganisation of the fibres) appears to be minimal. Except for low numbers of individuals infesting the myosepta, no microbes were observed on the fibres until decomposition had progressed to the point at which irregular taphonomic banding had developed (see above). At this point, the microbes were restricted entirely to the fibre's external surface; none having invaded the sarcomeres. Only when the actin and myosin filaments were no longer discernible, the organelles had been destroyed, and the carcass displayed signs of extensive decay, did microbial populations increase dramatically. Even at the termination of the experiments (73hrs postmortem), microbes had neither populated ruptures in the fibres, nor had invaded (i.e. pseudomorphed) the sarcomeres.

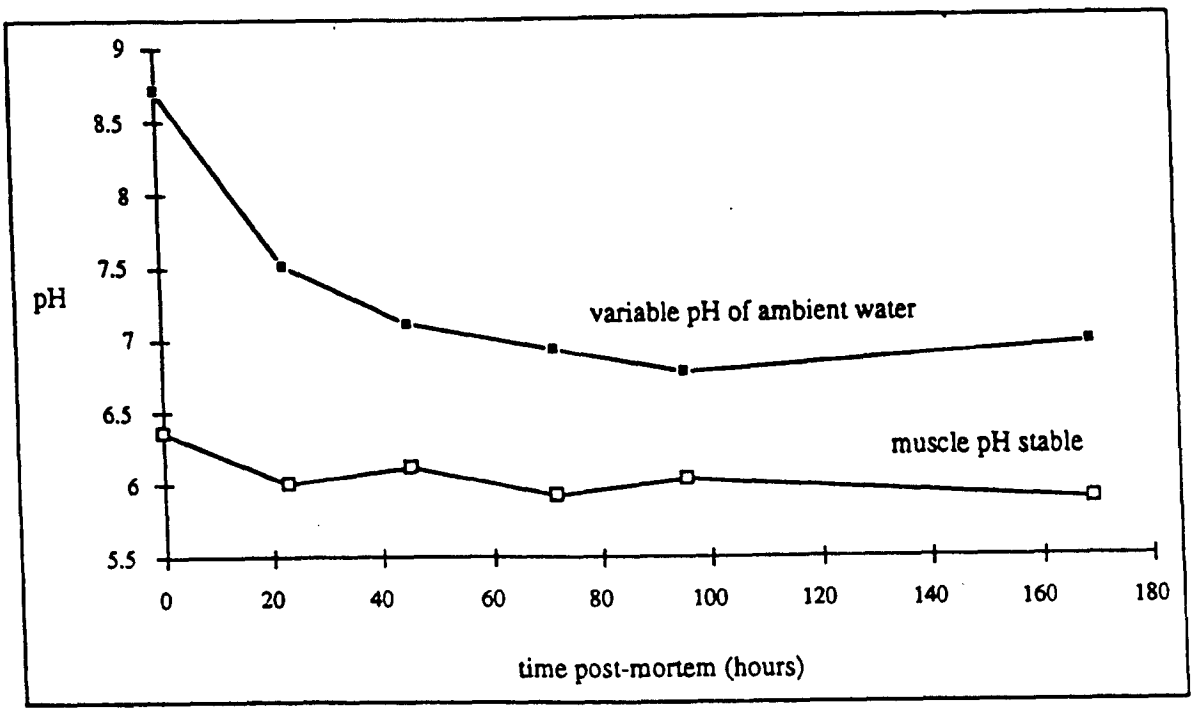
Interpretation of events: At least up until the point at which microbes are relatively abundant on the external surface of the muscle fibres, decay appears to have been dominated

by autolysis and osmotic stresses. The proliferation of bacteria thereafter is presumably a consequence of the increase in their ease of access to the muscle fibres from the external environment along ruptures in the body wall.

Berner (1968a, fig. 1) and Briggs and Kear (1993b, fig. 6) have demonstrated microbial fermentation to be associated with dramatic and rapid changes in pH. Initially, there is a sharp drop in pH associated with the production of fatty acids and CO_2 (Parkes and Senior, 1988). This is followed by a more gradual rise in pH which results from the release of ammonia and then trimethylamine from amino acids derived from proteins (Berner, 1968a, p195). The subordinate role of microbes in the initial stages of striated muscle decomposition described above, is supported by the relative stability of the pH of simultaneously dissected samples of skeletal muscle for at least the first 170hrs of decay (text fig. 6.4). These measurments (and those presented in Chapter 8) were made by dissecting blocks of muscle (approximately 5 x 10 x 10mm) from the relavent depth within the fish and thoroughly grinding them in a small vessel prior taking a reading with a fine-tipped pH probe. The reading was taken after three or four minutes to ensure it had stabilised.

Fossil microbes: The most immediately obvious evidence of decay in fossilized soft tissues is the presence of mineralized microbes. In muscle from the Romualdo Member, microbes are rare except in certain samples where they (much like in the taphonomic experiments) infest the outer surface of the sarcolemma and pseudomorph the myoseptum.

Microbes similarly infest only the outer surface of muscle fibres in many other deposits (e.g. the Crato Formation, the Portland Roach, the Lower Oxford Clay, and the Glencartholm Volcanic Beds, see Chapter 5). However, in Eckfeld Maarlake, the Lombardische Kieselkalk Formation, and the Gullane shrimp bed (see Chapter 5), the sarcomeres themselves are pseudomorphed by micro-organisms. On the basis of the experimental evidence presented above, it would appear that this is only possible after a prolonged period of decay (which is beyond the time that the procedure of wet specimen preparation adopted in the present study is effective; see Appendix 3iiia). This suggests that phosphatization in some deposits was considerably slower than in others.



Text figure 6.4: The stability in the pH of striated fish muscle (dissected from ≈5mm beneath the dermis of *Gadus* sp.) during the first 170 hrs of decay. In contrast, the pH of the ambient seawater (in support of Berner, 1968a) drops rapidly (carcass to water ratio =1:5). This suggests the initial decay of striated muscle in fish not to be controlled by microbes.

6.3 A SEMI-QUANTITATIVE ESTIMATE OF THE TIMING OF PHOSPHATIZATION OF STRIATED MUSCLE IN FISH FROM THE ROMUALDO MEMBER

Hypothesis: By comparing the morphology of progressively degraded striated fish muscle (decayed under a variety of controlled conditions) with fossilized striated fish muscle from the Romualdo Member, it should be possible to estimate both the timing of mineralization and something of the palaeoenvironmental conditions of the Romualdo Lagoon. Only at the point at which the fossil soft tissues were phosphatized and the environmental conditions of the Romualdo Lagoon are perfectly mimicked, will all of the subcellular components of the experimental muscle simultaneously resemble those of the fossil material. At all other times and under all other conditions, the various subcellular components of the muscle will not simultaneously resemble those of the fossil muscle.

6.3.1 EXPERIMENTAL CONDITIONS

Except for those differences given below, the experimental conditions adopted were identical to those described in Section 6.2.1.1.

Atlantic Cod were decayed under a variety of conditions to investigate the effects of salinity and temperature on the rate and style of skeletal muscle decay. In one set of experiments, the salinity was kept at 3.5% and decay monitored at a variety of temperatures (4^0C, 17^0C, 24^0C). In an alternative set of experiments, the fish were placed in decay vessels maintained at 17^0C (the temperature estimated for the bottom waters of the Romualdo lagoon, see Section 2.5.4) containing either freshwater, half normal marine (1.8%), normal marine (3.5%), twice normal marine (7%), or three times normal marine (10.5%) water. Each vessel was innoculated with 10cc of water taken from previous taphonomic experiments of compatible salinity. Muscle samples were removed at the time of death and at the following intervals postmortem (given in hours/minutes) - 17/0, 28/30, 42/0, 54/0, 67/15, 93/0, 121/30, 146/15, 164/15, 193/35, 217/0, 260/0, 307/15, 364/0, 408/30, 475/0 - and compared blind on the TEM with 'average' fossil muscle (see below).

COMPARISON STANDARDS (or 'average' fossil muscle): Sections 4.2.2.1 and 6.2.1.2 respectively demonstrated that even within an individual fish, skeletal muscle may have a number of different preservational styles, and may exhibit some *minor* variations in the extent of decay. Comparisons with experimentally decayed muscle therefore necessitates each component of the fossil muscle to be defined by a *single* specimen which displays the 'average morphology' of that component from all of the fossil muscle examined in the coarse of this study. Where preservational biases limit the number of examples of certain components which are preserved (e.g. mitochondria, T-tubules, and sarcolema), the standards are based on only a few tens of specimens. The standards for nuclei, sarcomeres and the connective fibrils however, are based on several tens and even hundreds of examples. For the striated fish muscle of the Romualdo Member I nominate: figure 4.20 for mitochondria; figure 4.17 for nuclei; figure 4.7 for sarcomeres (i.e. when >75% of the sarcomeres in each fibre are ruptured along their M-lines); figure 4.14 for the connective collagen fibrils at the terminal ends of each fibre; figure 4.24 for the sarcoplasmic reticulum

and the system of T-tubules; and figure 6.20 for the sarcolemma. Note that since all the structural components of striated muscle are rarely preserved in a single muscle fibre, each of the nominated representative is from a different specimen.

CONFIDENCE LIMITS: So that the results of this experiment may be compared in a meaningful way with those of others (e.g. Martill and Harper, 1990; Briggs and Kear, 1993a), I outline below the assumptions made and the limitations of my estimate:

Assumptions:

1) I assume the rate and sequence of autolytic decay in skeletal muscle to be the same in all bony fishes exposed to the same ambient conditions. Certainly, I was unable to detect any significant difference in the rate of decay of skeletal muscle between similarly sized specimens of cod and mackerel.

Allison (1990b) has recorded differences in the style and rate at which organisms of the same class decompose. These variations resulted from differences in the architectural design, the composition, and the quantity of connective tissue present within individual organisms, and are therefore irrelevant when considering the decomposition of a single tissue (e.g. skeletal muscle) over relatively short time intervals.

2) I have assumed the dermis of Atlantic Cod to be analogous to that of *Rhacolepis* and *Notelops* in terms of the speed at which microbes, water, and ions may invade the underlying tissues. The scales of the two fossil taxa are however, considerably larger and more densely mineralized than those of cod, and thus this assumption relies on the premise that differences in the numbers and architecture of scales does not affect either the osmotic properties of the dermis or the rate at which microbes may gain access to the underlying tissues.

3) I assume decay of the fossil fish occurred entirely at the sediment surface, and that the fish containing phosphatized soft tissues did not either: a) float in surface waters of a different temperature prior to their descent into the hypolimnion, or b) decompose in an environment which may have significantly reduced their rates of decay (e.g. an extreme pH).

It is difficult to comment on the validity of the latter assumption, but the former is substantiated to some extent by taphonomic evidence (see Section 2.5.3).

4) I assume that any differences in the level of oxygenation that existed between the experimental conditions (partially sealed vessels with a limited volume of water) and those of the Romualdo Lagoon (semi-restricted, reducing microenvironment, see Section 2.5.4) are irrelevant to the rate of decay of the muscle located 5mm beneath the dermis of the fish.

The effect of oxygen supply on microbial respiration has been the centre of some controversy (see Hecht, 1933; Zangerl and Richardson, 1963; Foree and McCarty, 1970; Zangerl, 1971; Curtis, 1980; Benner *et al.*, 1984; Westrich and Berner, 1984; Plotnick, 1986; Allison, 1988c; Emmerson and Hedges, 1988; Cannfield, 1989; Kidwell and Baumiller, 1990; Allison and Briggs, 1991b; Briggs and Kear, 1993b). However, little is known of the effects of oxygen supply on the rate of non-microbial decomposition. Since there is some evidence (see Jørgenson, 1977; Allison, 1988e, p151; Kidwell and Baumiller, 1990, fig. 6; Allison *et al.*, 1991, Briggs and Kear, 1993b, fig. 5) to suggest that due to the surface area/mass ratios of large carcasses, and the enormous oxygen demands of aerobic respiration, fish of 30cm length probably undergo anaerobic decomposition even in oxygenated waters, the concentration of dissolved oxygen in the surrounding water may not be particularly important. I do however acknowledge that the concentration of dissolved oxygen may greatly effect the osmotic behaviour of the decomposing organisms (Briggs and Kear, 1993b).

5) The composition of the interstitial waters of the Romualdo Lagoon in which phosphatization took place are not precisely known. Nevertheless, it is likely that the high porosity of the sediment (85%) would have permitted some diffusion between the carcasses, the interstitial fluids, and the overlying water column (see Manheim, 1970). In contrast, the taphonomic experiments described above were run in an extremely limited volume of water (a carcass to water ratio of 1:5). Herrero (1983) has demonstrated that such conditions may subdue or even inhibit the activity of microbes by limiting the availability of dissolved nutrients and allowing toxic metabolites to build-up (e.g. ammonia and H_2S). In agreement with Kidwell and Baumiller (1990, p259), I have assumed the effects of these processes to

have been greatly reduced in my experiments by the diffusion of the toxic metabolites into the atmosphere.

6) I assume decay in every component of the fossil muscle to have been halted simultaneously by mineralization. There is some evidence (see Briggs and Kear, 1993a) to suggest that even very 'light' mineralization may be sufficient to prevent further decomposition. Thus, my estimate for the timing of mineralization refers to the point postmortem at which phosphates were first precipitated in the soft tissues. I therefore assume that despite having experienced a period of recrystallization, decay was halted in soft tissues replaced by inorganic microspheres at the same time as those tissues which are pseudomorphed by microgranular apatite. That is, further decomposition was prevented in tissues replaced by inorganic microspheres by the precipitation of the amorphous precursor phase (see Section 3.3.5).

Limitations:

1) The results presented below refer exclusively to the timing of phosphatization of muscle located ≈5mm beneath the dermis of fish. Skeletal muscle situated at greater depths *may* decay more slowly due to its relative isolation from the external environment and microbes. Furthermore, assuming an external source of phosphorus (see Chapter 7), the timing of mineralization of deeper muscle may also be affected by the greater thickness of tissue over which ions must diffuse to the site of mineralization. This is supported by subtle differences in the extent of decay exhibited by different samples of muscle from individual fossil fish.

For the same reasons, the estimate presented here can't necessarily be extrapolated with complete confidence to other organs within the fossil fish. For example, it is clear from taphonomic experiments that the alimentary tracts of fishes decompose faster than skeletal muscle, and yet are often more perfectly preserved in the fossil fish.

2) Since some organisms (e.g. ostracodes and copepods) within the Romualdo Member are preserved quite differently to the skeletal muscle of the fish (see Chapter 4), it is unlikely that they were mineralized by the same process or at the same rate. It would therefore be prudent to apply the estimate given below only to those organisms replaced by inorganic

microfabrics. No attempt is made to estimate the timing of mineralization of soft tissues infested by microbes.

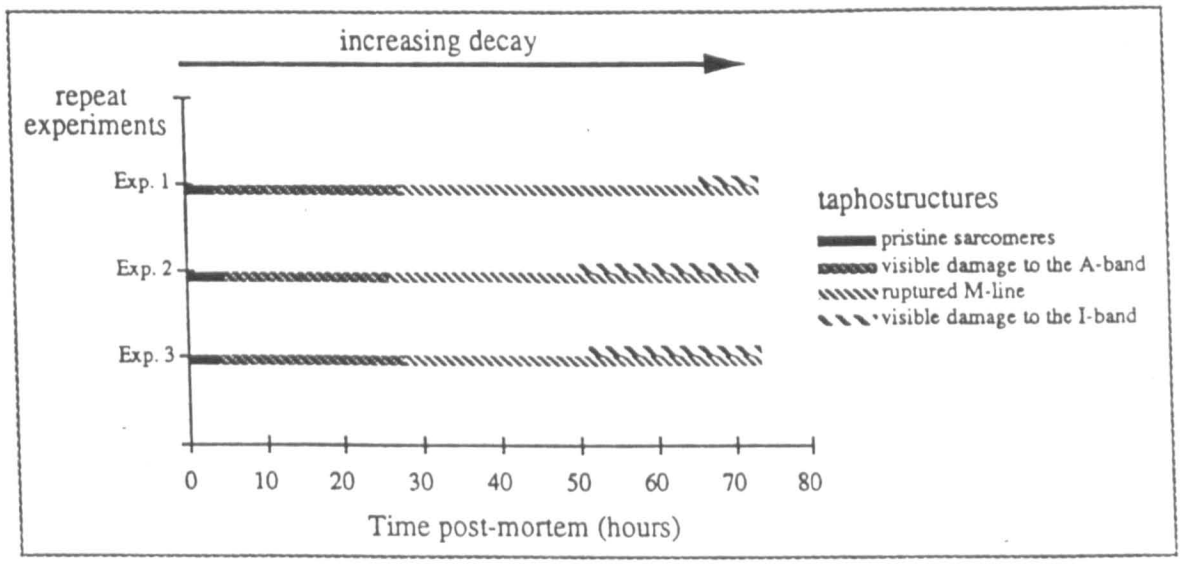
3) Section 6.2.1.2 demonstrated that inorganically replaced fish muscle in many deposits displays a similar level of decomposition to skeletal muscle in the Romualdo Member. This suggests phosphatization in these cases to have taken place at *similar* times postmortem. Unfortunately however, since the estimate given here is based on specific samples of muscle from the Romualdo Member, it may only be applied with *absolute* accuracy to this deposit. The timing of mineralization in other deposits may only be more precisely constrained by performing an independent comparative exercise. These are extremely time consuming and require a detailed knowledge of the preservation of the fossilized soft tissues.

6.3.2 RESULTS

The results of these experiments are presented in the form of isotaphytes. Isotaphytes may be defined as lines which join points of equal morphology on a graph whose X axis is time postmortem, and whose Y axis is an environmental parameter. In the present study, the isotaphytes define times at which specific components of skeletal muscle at different temperatures or salinities attain a morphology identical to that of the comparison standards (see Section 6.3.1). To the right of each isotaphyte, corruption of the component's morphology (due to decomposition) has surpassed that displayed by the fossil material, whereas to the left of each isotaphyte, the opposite is true. Because isotaphytes are based on morphological data, they are not strictly lines of equal decay, but rather the attainment of equimorphology. For example, due to the osmotic stresses imposed on organelles exposed to elevated or reduced salinities (see Section 6.2.1.2), the same morphology may be attained in different ambient conditions at a variety of times postmortem, and be caused by very different taphonomic processes.

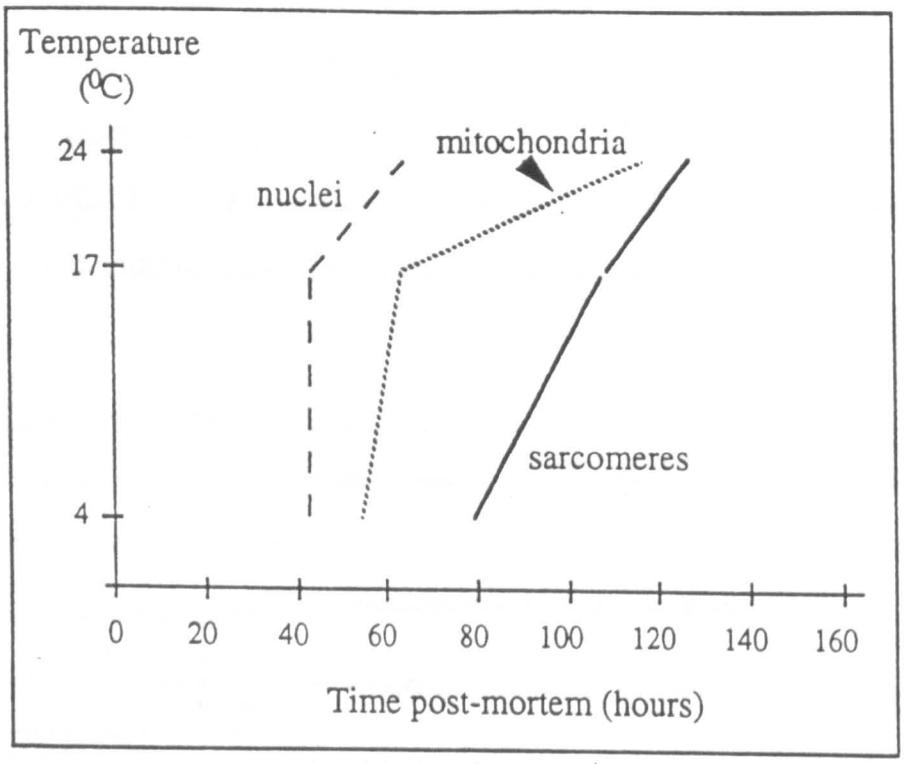
Each point on a isotaphyte defines the time at which 75%+ of that particular component of the experimental muscle attained (or surpassed) the morphology of the same component in the fossil muscle. These points represent the average of three simultaneously dissected samples. Despite the subjective manner with which each isotaphyte is plotted, blind plots of the time over which certain distinctive taphostructures in skeletal muscle occur, are

remarkably reproducible. Repeat experiments (run at 20^0C and 3.5% salinity) give a *maximum* variation on the mean of 8 hours (although for the majority of time it is considerably lower - typically <3hrs) at least for the first 73 hours of decay (text fig. 6.5). Therefore, assumptions and limitations in mind (see Section 6.3.1), I believe the results presented below provide a realistic estimate of the timing of mineralization in the Romualdo Member.

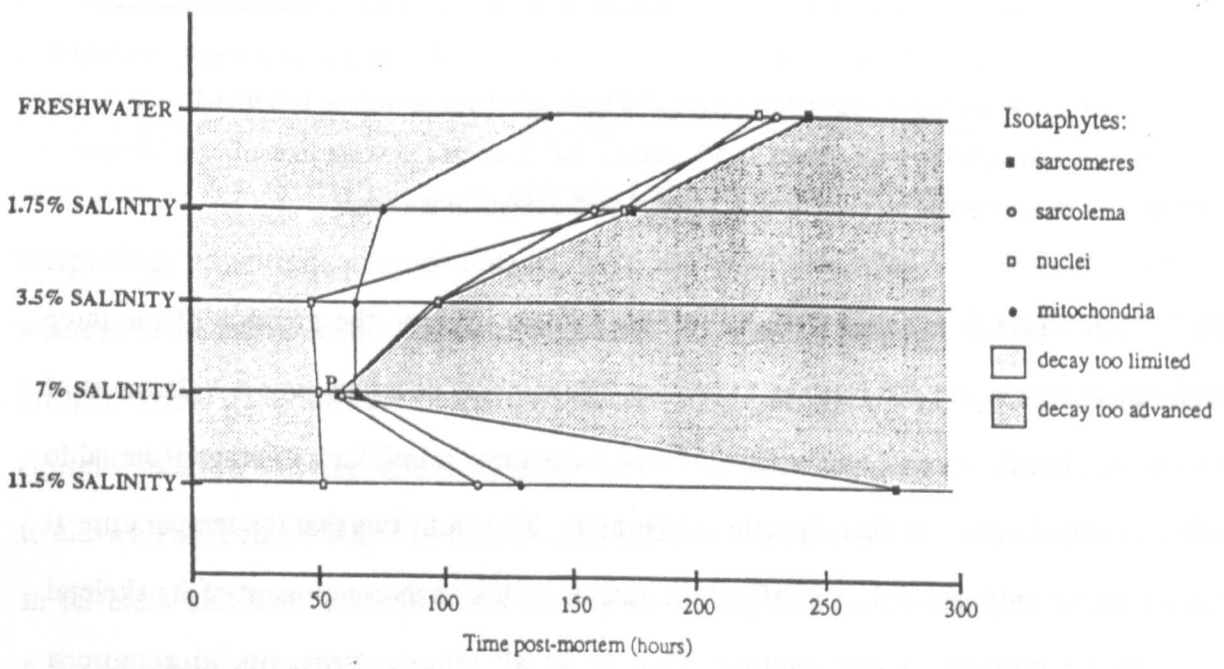


Text figure 6.5: The timing of appearance of specific taphostructures in skeletal fish muscle (*Gadus* sp.) from three simultaneously run experiments (3.5% salinity, 20^0C, carcass to water ratio of 1:5). The period over which each taphostructure persisted is demonstrated by the length of the lines.

TEMPERATURE EXPERIMENTS: For a given salinity, the position of the three isotaphytes in text figure 6.6 relative to one another remains constant over a wide range of temperatures, but the rate of appearance of taphostructures mimicking average Romualdo Member material increases sharply with temperature. This indicates that the temperature at which a fish decomposes does not affect the rate at which each component of its skeletal muscle decays relative to one another. That is, at all temperatures, nuclei acquire a morphology the same as those in the fossil specimens before mitochondria and the sarcomeres do.



Text figure 6.6: The effect of temperature (3.5% salinity, carcass to water ratio of 1:5) on the rate at which 75%+ of the nuclei, mitochondria and sarcomeres of Recent striated fish muscle (*Gadus* sp.) acquire a morphology identical to that of fossil striated fish muscle from the Romualdo Member. See text for details.



Text figure 6.7: The effects of salinity (17⁰C, carcass to water ratio of 1:5) on the rate at which 75%+ of the nuclei, mitochondria, sarcolemma and sarcomeres of Recent striated fish muscle (*Gadus* sp.) acquire a morphology identical to that of fossil striated fish muscle from the Romualdo Member. Error bars are based on text figure 6.5. The salinities and times over which phosphatization could not have taken place are shaded. See text for details.

SALINITY EXPERIMENTS: At a set temperature, the rate at which the various components of skeletal muscle acquire a morphology identical to that of 'average' muscle in the Romualdo Member, varies enormously for different salinities (text fig. 6.7). There is however, a general decrease in the rate at which each component assumes the morphology of its counterpart in average muscle from the Romualdo Member from a maximum at ≈7% salinity, to a minimum at higher and lower salinities. That is, autolytic and osmotic decomposition of striated muscle is at its most rapid in slightly hypersaline water.

Assuming a temperature of 17^{0}C for the bottom waters of the Romualdo Lagoon (see section 2.5.4), the superimposition of the isotaphytes for mitochondria, nuclei, sarcolemmas, and sarcomeres at point 'P' in text figure 6.7, suggests phosphatization to have ceased decay at 55 hours postmortem, and the bottom waters of the Romualdo Lagoon to have been hypersaline (≈7% salinity). Rapid mineralization and a hypersaline environment is strongly supported by palaeoenvironmental and taphonomic evidence (see Sections 2.5.1 and 2.5.4.).

6.3.3 DISCUSSION

TIMING OF MINERALIZATION: There is a considerable difference between the timing of phosphatization estimated here and that of less than 5hrs given by Martill and Harper (1990) for the gills of fish from same deposit. This discrepancy may stem from a number of factors. Firstly, assuming an external source of phosphate ions (see Chapter 7), peripherally located organs such as gills are likely to become supersaturated with apatite faster than more internally positioned tissues (e.g. skeletal muscle). It is therefore likely that gills would have been mineralized more rapidly than skeletal muscle. Secondly, in contrast to the situation in skeletal muscle where inorganic processes of mineralization dominated, the gills of fish from the Romualdo Member are characterized by microbial infestation (see Section 4.2.2.1). Therefore, both the mechanism of nucleating apatite and the source of phosphate ions were probably different for the two tissues (see Chapters 3 and 7 respectively), and thus the speed of mineralization is unlikely to have been the same. Thirdly, and perhaps most significantly, the experimental conditions adopted by Martill and Harper (1990) are questionable. They immersed *freshwater* fish (*Salmo gairdneri*) in normal

marine water and based their estimate of the timing of mineralization on the development of a single feature - the collapse of secondary gill lamellae. However, many ichthyologists argue the fish of the Romualdo Member to be marine taxa (e.g. see Schaeffer, 1947; Silva Santos and Valenca, 1968; Martill, 1988), and palaeoenvironmental (see Section 2.5.4) and microtaphonomic data (see Section 6.3.2) suggests them to have decomposed in hypersaline water. Furthermore, the gill apparatus of *Rhacolepis* and *Notelops* (see text figure 4.7) is considerably more robust (for use in ram ventilation, see Section 4.2.2.1) than that of Rainbow Trout (*Salmo gairdneri*). Rainbow Trout are therefore unlikely to provide an accurate taphonomic analogy. Indeed, gills may be an altogether inappropriate tissue on which to base estimates of the timing of mineralization. Repetition of Martill and Harpers' (1990) experiments indicate that once collapsed, secondary gill lamellae may remain stable in a collapsed position for extensive periods (several days). Martill and Harpers' (1990) results can therefore only be taken to be the absolute minimum time elapsed between death and mineralization.

A greater discrepancy exists between the timing of mineralization estimated here, and that determined experimentally by Briggs and Kear (1993a) for the muscle of small shrimps (*Crangon crangon* and *Palaemon* sp.). In their actualistic experiments, mineralization commenced within 2 weeks of the shrimp's death and increased for at least 2 to 6 weeks thereafter. Relative to my own estimate for the timing of phosphatization, this represents a delay in mineralization of at least 12 days. Briggs and Kear (1993a) proposed a time lapse of 2 weeks was necessary between death and mineralization to permit phosphorus to be released by microbes into the water from the shrimp's carapace. In the Romualdo Lagoon, it is unlikely that such a delay in mineralization occurred since concentrations of dissolved phosphorus in the sediment were already elevated prior to the introduction of the dead organisms (see Chapter 7). The two different estimates may therefore refer to two distinct (but related) processes of mineralization.

RATES OF MORPHOLOGICAL CHANGE:

Temperature experiments: Despite representing essentially autolytic decay, the slope of the isotaphytes in text fig. 6.6 suggest the rate of decay of skeletal muscle to increase with

temperature. This corroborates the findings of most taphonomists regarding microbially-dominated decomposition (e.g. Schäfer, 1972; Kidwell and Baumiller, 1990). This is not surprising since all chemical reactions including autolysis are temperature dependent.

Salinity experiments: Above and below ambient salinities of 7%, the rate of morphological change of every element of skeletal muscle is reduced (see text fig. 6.7). The causes of these trends are discussed below:

The slow rates of morphological change of organelles decayed in solutions of low salinity (i.e. below 7%) probably reflect the closer match in solute concentration of the physiological fluids (≈0.9% salinity) with these solutions, and thus the minimal level of osmotic stress. Indeed, a small net influx of water may actually prolong the originally globose outline of the organelles. Slower rates of sarcomere and sarcolemma decay at salinities below 7% almost certainly result from a reduction in the severity of rigor mortis. Postmortem contraction in muscle fibres is normally driven by Ca^{2+} controlled conformational changes in an actin associated accessory protein - troponin (Alberts *et al.*, 1989, p622). This, however, may be inhibited in solutions with a salinity less than that of physiological fluids, by a reduction in the concentration of Ca^{2+} resulting from an osmotic influx of water.

The rate of disintegration of striated muscle is also reduced in hypersaline solutions (10.5% salinity). In the case of the organelles, this is somewhat surprising since one would predict the mitochondria and in particular the nuclei to experience extensive dehydration, and therefore display rapid changes in their morphology. It is not clear why this does not take place. Part of the explanation may be that this process is counteracted by the diminished reactivity of the tissue's indigenous lysosomal enzymes under high salinities. This is more effective in mitochondria than nuclei since the latter are more susceptible to osmotic stress.

Reduced rates of disintegration of the sarcomeres in hypersaline solutions probably results from the interference of Ca^{2+} binding sites on the troponin complex (see above) by other ions, and therefore (as in hypotonic solutions) a reduction in the severity of rigor mortis. Although of greater salinity than physiological fluids, the rapidity with which skeletal muscle decomposes in solutions of 7% salinity, suggests this concentration not to have been sufficiently great to have initiated the inhibitory processes described above.

Interestingly, there is a corresponding change in the abundance of microbes infesting the outer surface of the muscle samples. Microbe numbers reach a maximum at 7% salinity and diminish rapidly with at higher and lower salinities. Although Allison (1988c, 1990b) has demonstrated *microbial* decomposition to be slower in freshwater than in sea water, I do not consider the relative abundance of microbes in my experiments to have been the cause of the differences in the rate of morphological change observed in the muscle. Indeed, I do not believe the numbers of microbes to be *directly* related to the ambient salinity. Instead, their relative abundances are more likely to be a consequence of the salinity dictated rates at which the muscle fibres undergo autolytic and osmotic degradation (see above). When autolytic and osmotic decomposition is rapid, the microbes may gain access to the muscle fibres from the external environment more rapidly along ruptures in the muscle fibres than when decomposition is slower.

6.4 CONCLUSIONS

1) Microtaphonomic studies are crucial to an understanding of the processes and timing of soft tissue fossilization. They permit the quantity of information obscured, destroyed, and/or not preserved by the mineralizing process to be established.

2) The sequence and timing of appearance of taphostructures in skeletal muscle decomposed in the absence of microbes are predictable over a wide range of environmental conditions. The rate of decomposition is dictated largely by the relative activity of indigenous enzymes, the severity of rigor mortis, and the level of osmotic stress.

3) Phosphatization of soft tissues is an extremely rapid postmortem event. Making certain reasonable assumptions, I estimate the skeletal muscle of fish from the Romualdo Member to have been phosphatized within ≈55hrs of death. Other tissues, particularly those located deeper within the carcasses, may have taken longer to be phosphatized due to the greater thickness of tissues through which dissolved phosphorus needed to diffuse from the external source.

4) The style and sequence of decomposition of striated muscle in microtaphonomic experiments confirm the bottom waters of the Romualdo Lagoon to have been hypersaline.

5) Microtaphonomic experiments suggest that at the time of phosphatization, many of the constituent biomolecules of skeletal muscle in fish from the Romualdo Member would have been at least partially intact. Membranes would have been coherent, actin and myosin filaments still discernible (although somewhat disorganised), and collagen would still have retained its characteristic pattern of banding. In contrast, the chromatin of nuclei would have suffered extensive degradation by the time they were fossilized.

6) Phosphatized striated fish muscle from a number of lagerstätten display comparable levels of decay to muscle from the Romualdo Member. It is therefore reasonable to assume these to have been phosphatized at *roughly* the same time postmortem.

7) Differences in the preservational style (and therefore mechanism of mineralization) and/or the source of phosphorus for different organisms even within the same deposit, suggest the timing of soft tissue phosphatization to be taxonomically influenced. Furthermore, taphonomic experiments suggests fossil muscle replaced by inorganic microfabrics (e.g. Romualdo Member) to have been fossilized more rapidly than muscle coated- (e.g. Portland Roach) or pseudomorphed by microbes (e.g. Lower Lias). The timing of phosphatization is extremely tissue-, organism-, and preservational style-specific.

CHAPTER 7
PHOSPHATIZED SOFT TISSUES: THE SOURCE OF PHOSPHORUS

7.1 INTRODUCTION

One of the most obvious requirements for the phosphatization of soft tissues is a source of calcium and phosphorus. In contrast to Ca^{2+} which is the fifth most abundant ion in sea water (≈4.1 x10^5 µg/litre), phosphorus is a biolimiting element and rarely exceeds a concentration of 90 µg/litre (Mason and Moore, 1982, Table 9.3). Although sea water is supersaturated with respect to apatite at this concentration (Dietz *et al.*, 1942), kinetic factors prevent it from precipitating (Atlas, 1975, p103). Apatite is even less likely to precipitate in fresh water systems because although calcium has a concentration of 15000µg/litre, phosphorus averages only 20µg/litre. In order to overcome these kinetic barriers, all models of postmortem phosphatization must incorporate a mechanism of elevating the concentration of dissolved phosphorus ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}) in solution above those recorded from contemporary marine and non-marine waters (Mason and Moore, 1982, Table 9.3).

This chapter examines the likely provenance of phosphorus for the phosphatized soft tissues of a number of deposits. The relative contribution of several possible sources of phosphorus in the Romualdo Lagoon are tested by mass balance calculations, and I present a model for the provenance of phosphorus in this deposit based on established models of phosphogenesis.

Although I recognise the importance of Ca^{2+} to the phosphatization of soft tissues, this ion is abundant in both marine and fresh water environments (Mason and Moore, 1982, Table 9.3). Its source is therefore not discussed here.

7.2 THE SOURCE OF PHOSPHORUS IN THE ROMUALDO MEMBER (refer to text fig. 7.1).

Any model concerning the source of phosphorus for the phosphatization of soft tissues in the Romualdo Lagoon must conform to a number of geochemical and palaeoenvironmental constraints. Five factors require particular attention. These are: 1) the fidelity and quantity of

soft tissues phosphatized in individual organisms, 2) the existence of gradients in the density of phosphatization within individual carcasses, 3) the total mass of apatite precipitated in the Romualdo Lagoon, 4) the difference in the extent of phosphatization between those organisms which remained at or close to the sediment/water interface, and those which sank into the substrate (see Section 4.2.1), and 5) the occurrence of phosphatized soft tissues in some but not all fish at individual localities.

Each of these is discussed independently below. For simplicity, I restrict my comments in this section only to the source(s) of phosphorus responsible for the phosphatization of soft tissues in the fish of the Romualdo Member:

1) The fidelity and extent of phosphatization: the exceptional precision with which the soft tissues of fish from the Romualdo Member are preserved (see Section 4.2.2.1) suggests Ca^{2+} and dissolved phosphorus to have been immediately available for precipitation. However, the fossilization of only a fraction of the soft tissues of most fish (see Section 4.2.1) implies this source to have been limited in size and to have become exhausted relatively quickly (or the process of mineralization to have been interrupted).

The most extensively phosphatized fish that I have examined (PRW/11) contained $160cm^3$ of soft tissues. Assuming: a) $1cm^3$ of phosphatized soft tissues freed of matrix to have a mass of 0.038g (calculated from acid digestions of specific volumes of soft tissues assuming 50% of all soft tissues are not recovered, see below), and b) apatite to have the simple formula - $Ca_5(PO_4)_3OH$ [a molecular mass of 502], the most extensively phosphatized fishes contain a mass of PO_4^{3-} of:

$$(160 \times 0.038) \times (95/502) = 1.15g \text{ (or } 0.38gP = 12258\mu M) \qquad \text{Calculation 7.1}$$

This mass, according to my estimate for the timing of mineralization of the soft tissues of fish in the Romualdo Member, was precipitated in the fish in just 55hrs (see Chapter 6).

2) The mass of apatite precipitated in the Romualdo Lagoon: Making the same assumptions as for calculation 7.1 (see above) and also assuming that: a) 10% of all concretions in the Romualdo Member contain a specimen of *Notelops* sp. or *Rhacolepis* sp. with phosphatized soft tissues (estimated from fieldwork and acid digestions), b) the average

volume of soft tissues preserved in each of these fish is $5cm^3$, c) the Romualdo Member contains at least 7 horizons of concretions each of which has a concretion every $1m^2$ (estimated from field work), and d) the Romualdo Lagoon covers an area of $17500km^2$ (an underestimate of the original extent, see Section 2.2); a conservative estimate for the mass of apatite deposited within the soft tissues of fish carcasses within the lagoon is:

$(7 \times 17500 \times 10^6)/10 \times (5 \times 0.038) =$ **2328 tonnes** Calculation 7.2

Any model of phosphatization must therefore be capable of producing a comparable mass of apatite.

3) Gradients in the density of phosphatization within fish: EDAX elemental maps (see Appendix 3i) of transversely sectioned fish frequently display gradients in the density of phosphatization of their soft tissues from a high at their peripheries just beneath the dermis, to a low at ≈7mm depth within the fish (fig. 7.1). These gradients are confirmed by atomic number absorption fluoresence (ZAF) correction analysis. In general, at 7mm or more beneath the dermis of fish and pterosaurs, the density of phosphatization is so limited that individual apatite crystals and crystal aggregates remain isolated from one another, the interstitial spaces being filled by large diagenetic calcites (fig. 7.2). As a result, many muscle fibres are unable to survive acid digestion (Martill *et al.*, 1992). Commonly a phosphorus gradient also exists across individual muscle fibres such that the central myofibrils are not mineralized (figs. 7.3 and 7.4).

4) Preferential phosphatization of organisms at certain levels in the sediment: In contrast to regions of active phosphorite formation today (e.g. the Peru/Chile continental shelf) where the concentration of phosphate ions increases from ≈3μM/PO_4^{3-} in the water column to several hundred μM/PO_4^{3-} at depths of around 50cm in the sediment (see for example Brooks et al., 1968; Sholkovitz, 1973; Baturin, 1972), phosphatized soft tissues most commonly occur (and reach their greatest development) in those fish from the Romualdo Member which did *not* sink below the sediment/water interface (i.e. bedding-plane parallel fish, see Section 2.5.3). This implies that only the top few centimetres of the sedimentary pile in the Romualdo Lagoon were conducive to the precipitation of apatite.

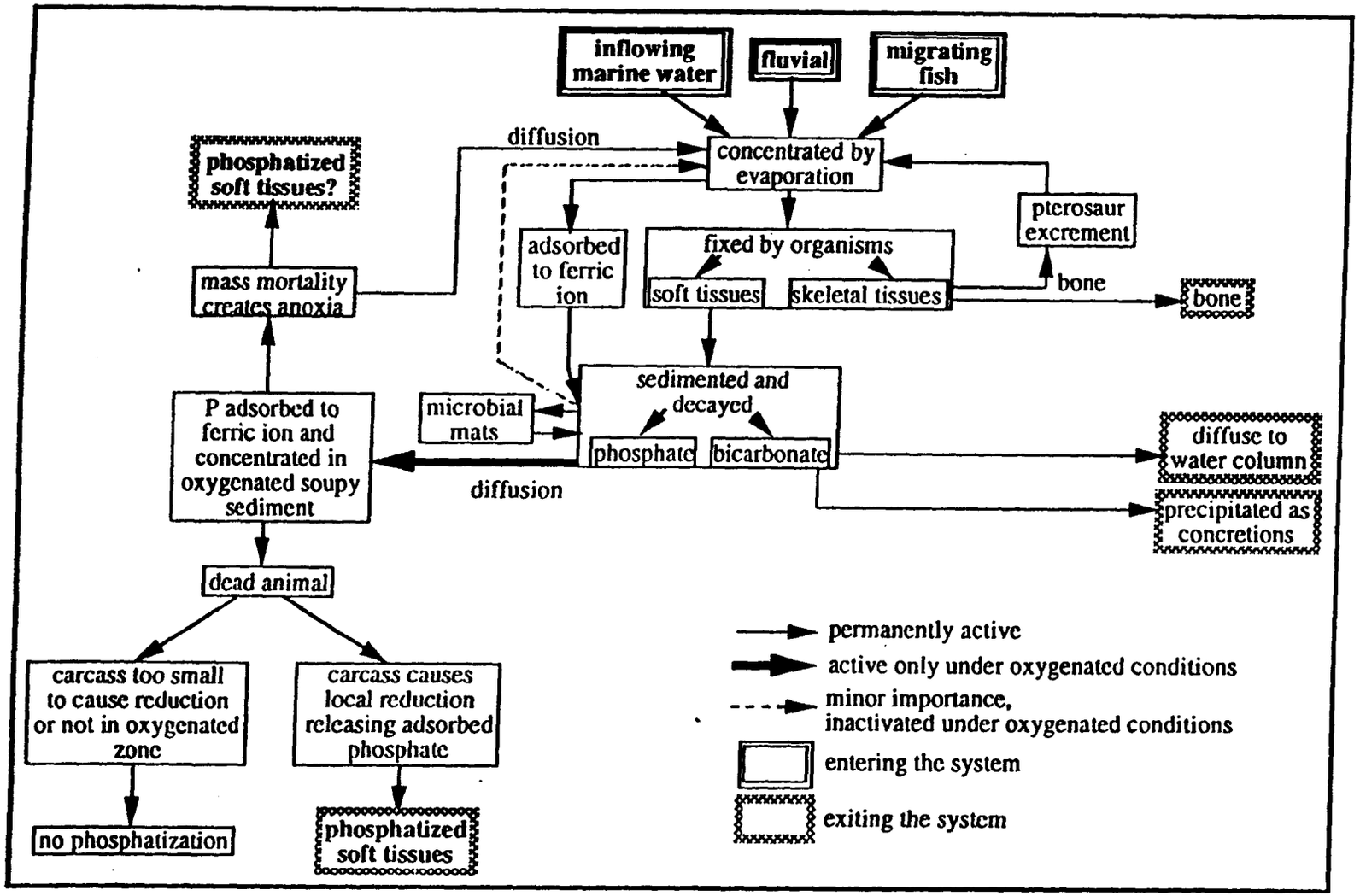
5) Differences in the extent of phosphatization of fish at individual localities: Even at a single locality, bedding-plane parallel specimens of *Notelops* sp. and *Rhacolepis* sp. from different concretionary levels contain different quantities of phosphatized soft tissues. This suggests that the quantity of phosphorus available to the decomposing fish varied with time. Any model of soft tissue phosphatization for the Romualdo Member must therefore be capable of encompassing these temporal variations.

7.2.1 POSSIBLE SOURCES

A number of different sources of phosphorus have been implicated in the postmortem phosphatization of soft tissues (see Section 1.6.1). These may be divided into two major groups: 1) those in which phosphorus was derived from the carcass undergoing mineralization (i.e. internal sources), and 2) those in which phosphorus was derived from a source other than the carcass undergoing mineralization (i.e. external sources). The relative importance of these two sources in the Romualdo Member is discussed separately below:

1) INTERNAL SOURCES: Phosphorus may have been derived from one or more of three internal sources. These are:

i) Body fluids: In one of the earliest studies of phosphatized soft tissues, Reis (according to Dean, 1902, p275) proposed the phosphorus responsible for the phosphatization of soft tissues in fish from the Solnhofen Limestone (see Section 5.2.5) to have been derived from their body fluids. Similarly, Schultze (1989) believed the soft tissues of fish from the Cordillera de Domeyko to have been phosphatized by phosphorus supplied by their own body fluids. Certainly, blood and other body fluids are supersaturated with respect to apatite (Lehninger, 1983, pp111-112), and therefore represent a large and potentially accessible source of phosphorus to decomposing organisms (or as in Schultze's, 1989 model - live organisms). Apatite is prevented from precipitating in the body fluids of living organisms by certain kinetic barriers and the presence of nucleation inhibitors (Lehninger, 1983, pp111-112). These inhibitory safeguards are likely to persist for a considerable length of time postmortem (see Section 8.3.1). Body fluids are therefore



Text figure 7.1: The phosphate budget for the Romualdo Lagoon

unlikely to have played an important role in the phosphatization of soft tissues in the fish of the Romualdo Member.

ii) Phosphate-rich gastric contents: With the exception of Reis (1893, 1895), the potential of gastric contents as a source of phosphorus for the phosphatization of soft tissues has not been explored. The gastric contents of fish such as *Rhacolepis* and *Notelops* which preyed on fish fry and crustaceans (both of which are particularly rich in phosphorus, see Vinogradov, 1953), are likely to represent a potentially large and immediately available source of phosphorus postmortem. The digestive tract, its contents, and organs in close proximity to the alimentary canal would be most susceptible to phosphatization by this source. Frequently, cololites and tissues of the alimentary tract of *Rhacolepis* and *Notelops* are preserved in even greater detail than the muscle of these fish (see Section 4.2.2.1). This suggests that relative to other tissues, these organs were fossilized extremely rapidly. It would therefore appear that some phosphorus from the alimentary tract was made available postmortem to the surrounding tissues of these fish. However, the rarity of fossilized examples of closely associated organs such as the kidneys, liver, and gas bladder (see Section 4.2.2.1), suggests this source to have been only *very* locally important and not to have been involved in the phosphatization of tissues beyond the alimentary tract (i.e. the gills and dermis).

iii) Microbial decomposition of the soft tissues: Phosphorus is relatively abundant in the soft tissues of most organisms (see Vinogradov, 1953), and therefore every carcass constitutes a significant reservoir. Microbes are essential to the release of this phosphorus (see Sholkovitz, 1973; Berner, 1974, 1977, 1980; Froelich *et al.*, 1979; Lucas and Prévôt, 1981, 1984; Benmore *et al.*, 1983; El Faleh, 1988; Prévôt *et al.*, 1989; Hirschler *et al.*, 1990a, 1990b; Briggs and Kear, 1993a) and have been invoked in the phosphatization of thoroughly decomposed soft tissues in a number of deposits (e.g. Eckfeld Maarlake [Micklich and Wuttke, 1988], Lake Odernheim [Willems and Wuttke, 1987]). Martill (1988) proposed such organically-bound phosphorus to have been *one* of several sources available to the decomposing fish of the Romualdo Member. He suggested this phosphorus to have been released from the tissues by microbes, and to have been subsequently precipitated either in the short-lived supersaturated microenvironment induced

by the metabolising microbes (Martill, 1988), or as a discrete entities within the microbes (Martill, 1991).

Certainly, the mass of phosphorus bound in a said volume of Recent fish muscle is similar to that locked in the same volume of fossil fish muscle. The muscle of Recent fish contains a mass of 1.85 x 10^{-3}gP/cm^3 of organically-bound phosphorus (based on a content by mass of 0.94%P, averaged from data given by Vinogradov, 1953, Table 296), whereas fossil fish muscle from the Romualdo Member contains 2.37 x 10^{-3}gP/cm^3 (based on the assumptions made in calculation 7.1).

Even if all of the phosphorus organically-bound in the fossilized muscle of fish from the Romualdo Member had not been released by microbes, there is more than sufficient organically-bound phosphorus elsewhere in the carcasses (i.e. in those tissues which are not themselves fossilized) to have made up the 'short-fall'. However, despite the apparent potential of organically-bound phosphorus to provide all of the necessary phosphorus for the phosphatization of soft tissues in the fish of the Romualdo Member, this was probably not released until some time after the mineralizing event. There exists a delicate balance between the quantity of phosphorus released from soft tissues by microbes, and the quantity of information destroyed by the same process (Briggs and Kear, 1993a). The release of all of a tissue's phosphorus would require its complete decomposition. This is not consistent either with the exceptional preservation of soft tissues in the Romualdo Member fish, or the rarity of fossilized microbes in these tissues (see Section 4.2.2.1). It is also unlikely that the phosphorus responsible for mineralization was derived from the decay of those organs that are not preserved in these fish. This would require the complete decay of these organs within 55 hrs of death. Taphonomic experiments indicate that this is unlikely (see text figs. 6.4-6.7).

Therefore, although internally-derived sources of phosphorus may have made minor contributions to the phosphatization of soft tissues in the Romualdo Member, they do not satisfy all of the constraints outlined in Section 7.2. In particular, assuming an internal source of phosphorus, it is difficult to account for the exceptional preservation of the soft

tissues, gradients in the density of phosphatization within individual fish, and differences in the extent of mineralization of different fish.

2) EXTERNAL SOURCES: The occurrence of gradients in the density of phosphatization of soft tissues within the fossil fish and across individual muscle fibres (see Section 7.2) is consistent with dissolved phosphorus having infiltrated the carcasses from an external source. An external source of phosphorus is also consistent with the absence of certain internal organs in these fish (e.g. the kidneys). These organs were presumably too isolated from the source of phosphorus to become saturated with respect to apatite.

The mass of apatite precipitated in the soft tissues of fish from the Romualdo Lagoon (i.e. that in excess authigenic phases disseminated throughout the sediment) was large (≈2328 tonnes, see Calculation 7.2). This must have been derived either from: i) the water column, and/or ii) the sediment. Martill has proposed these two sources to have been responsible for the phosphatization of soft tissues in the Romualdo Member both independently (Martill, 1989a), and in combination (Martill, 1988). The merits of both sources are discussed independently below:

i) The water column: Martill (1989a) has proposed two mechanisms by which the concentration of dissolved phosphorus in the water column may have been elevated to the point at which it precipitated in the fish directly from solution. In the first, he (Martill, 1989a, fig.2) envisaged the bottom waters of the Lagoon to have become enriched in dissolved phosphorus through the microbial regeneration of phosphorus from fish killed in mass mortality events. These supersaturated brines were prevented from being diluted by the rest of the water column by a stubborn thermo/halocline.

Although the presence of a stratified water column can be demonstrated (see Section 2.5.4), and fish mass mortalities undoubtedly made an important contribution to the phosphate flux of the Romualdo Lagoon, mass balance calculations do not support Martill's (1989a, fig. 2) model. Assuming: a) the concentration of phosphorus in Recent fish muscle to be $1.8 \times 10^{-3} gP/cm^3$ (see Section 7.2.1), and b) the average volume of each fish killed in the mass mortalities of the Romualdo Lagoon to have been 1 litre (≈ a 25cm standard length

fish), the mass of apatite precipitated in the Romualdo Member (2328 tonnes of apatite = 144.3 tonnes of phosphorus) would require the decomposition of:

$(144.3 \times 10^{6}/1.8 \times 10^{-3})/1000$ = **80 million fish** Calculation 7.3

Although enormous numbers of fish may be killed in mass mortalities (Brongersman-Sanders, 1957) and the number of concretions in the Romualdo Member ($\approx 1.225 \times 10^{11}$) indicates that more than sufficient fish did die to account for the mass of apatite precipitated in the lagoon, the development of a sufficient saturation of apatite in the hypolimnion at *any one time* by this process is questionable (and impossible if only a "few thousand fish die" [Martill, 1989b, p5]). For example, only by assuming the hypolimnion to have been 10m thick (and to cover an area of 17500km^2), and all of the phosphorus from the 1.225×10^{11} fossil fish in the Romualdo Member to have been available *at the same time*, can the concentration of HPO_4^{2-} in the bottom waters reach those of the interstitial waters of modern phosphorite formation (5-220μM, Hartmann *et al.*, 1973, 1976; Sholkovitz, 1973; Golhaber *et al.*, 1977; Suess, 1981; Jahnke *et al.*, 1983). However, when considering that: a) the 10m$^+$ of sediment in which fish-bearing concretions occur represents a considerable period of time and therefore all the fish were not killed at once, b) phosphatization of soft tissues occurred more than once but the bottom waters of the Romualdo Lagoon were regularly mixed with the epilimnion (see Section 2.5.4), it is clear that the hypolimnion would have been incapable of maintaining the necessary concentration of phosphorus over the periods of time required. That is not to say that phosphorus released from decomposing fish was not important in the phosphatization of soft tissues in the Romualdo Member; it merely implies that it is unrealistic to concentrate organically-derived phosphorus in the massive volume of the hypolimnion.

Martill's (1989a, fig. 3) second model relying on the concentration of phosphorus in the hypolimnion suffers from similar problems. In this model ("pterosaur crap model"), he proposed phosphorus to have been derived from the dissolution of guano from surrounding pterosaur nesting grounds. Although it is conceivable that quantities of apatite far in excess of those necessary for the phosphatization of all of the soft tissues in the Romualdo Member could have been derived from guano, it is not clear how the phosphorus would have been

concentrated in the hypolimnion. Phosphorus is a biolimiting element with a short residence time (1.8×10^5 years, Mason and Moore, 1982, Table 9.3) and therefore rarely becomes concentrated in natural aqueous systems. Furthermore, Martill (1989a) did not make it clear how such a "supersaturated" (Martill, 1989a, p5) solution could be stabilized prior to the introduction of fish carcasses. Thermodynamic and kinetic calculations (Atlas, 1975, p101; Burnett, 1977; Baturin and Bezrukov, 1979; Nathan and Sass, 1981) predict apatite would have precipitated in the porewaters of the sediment and/or onto disseminated organics prior to building up to the levels envisaged by Martill (1989a).

ii) The sediment: Most workers agree (e.g. see Van Cappellen and Berner, 1988) that phosphorus is continually removed in large quantities from the water column and transported to the sediment surface. This is achieved either by: a) fixing it in the tissues and excrement of organisms, and/or b) adsorbing it to hydrous ferric oxides and hydroxides (Berner, 1973; Benmore *et al.*, 1983). The contribution of these two sources to the phosphate budget of the Romualdo Lagoon's sediment are discussed independently below:

a) Fixation by organisms: Phosphorus may be fixed in both the mineralized skeleton and soft tissues of organisms, and then be released to the sediment through their dissolution (Posner *et al.*, 1984) and microbial decay (El Faleh, 1988; Prévôt *et al.*, 1989) respectively. Skeletal tissues have only been demonstrated to be an important source of phosphate ions in a few regions of modern phosphogenesis (Suess, 1981; Van Cappellen and Berner, 1988); soft tissues are interpreted as the major source of phosphorus in all other cases (see for example Sholkovitz, 1973; Berner, 1974, 1977, 1980; Froelich *et al.*, 1979; Krom and Berner, 1984; Benmore *et al.*, 1983).

The pristine preservation of even the most delicate fish and pterosaur bones in the Romualdo Member suggests that as in most regions of active phosphogenesis (see above), biogenic apatites did not contribute significantly to the phosphorus budget in the Romualdo Lagoon, except perhaps when re-introduced into solution in the form of excrement (e.g. Martill, 1989a, fig. 3). The major source of dissolved phosphorus for phosphatization was probably that released by the microbial decay of disseminated organic detritus and large carcasses (such as fish killed in mass mortalities, Martill, 1988, 1989a) in the sediment (see text fig. 7.1). The productivity of the Romualdo Lagoon and therefore the flux of organic

material to the sediment surface is attested by the abundance and diversity of the ichthyofauna (see Maisey, 1991).

Martill (1988) proposed phosphorus liberated from disseminated organics in the sediment to have been one of three major sources (the others being the decaying carcass itself and the overlying water column) of phosphorus in the Romualdo Lagoon. He suggested that phosphorus had diffused directly up from the sediment into fish lying on the sediment surface. Dissolved phosphorus released from organic-rich sediments is known to diffuse back into the overlying water column (Elderfield *et al.*, 1981; Suess, 1981; Filipek and Owen, 1981; Froelich *et al.*, 1982), and some phosphorite concretions have been demonstrated to grow down into the sediment in response to the upward diffusion of phosphate ions (Burnett *et al.*, 1982). However, the rates at which these phenomena occur are not consistent with the flux of ions necessary for the mineralization of soft tissues in the Romualdo Member. For example, Burnett *et al.* (1982, p1617) give values of 10 $x10^{-9}$ to 200 $x10^{-9}\mu Mcm^{-2}sec^{-1}P$ for the accumulation of apatite concretions, and Suess (1981) 65 $x10^{-9}$ $\mu Mcm^{-2}sec^{-1}$ for the flux of phosphate from the sediments underlying the Peru-Chile upwelling zone (the most active region of phosphorite formation today). Therefore, by making the same assumptions as for calculations 7.1 and 7.2, and assuming: a) phosphate fluxes from the sediment in the Romualdo Lagoon to have been comparable to those recorded by Burnett *et al.* (1982, p1617), and b) *all* of the phosphorus diffusing up from beneath the carcasses to have been precipitated in their tissues; the phosphorus required for the mineralization of soft tissues in the most heavily phosphatized fish (PRW/11, which had a surface area $\approx 200cm^2$ in contact with the sediment) would in a conservative calculation take:

$(12258/200)/200\ x10^{-9} = 3 \times 10^{8}$ secs (=85125hrs) Calculation 7.4

My taphonomic experiments suggest that the soft tissues of fish from the Romualdo Member were phosphatized within 55hrs of death (see Section 6.3.2). Therefore, according to calculation 7.4, phosphorus would have had to diffuse through the sediment of the Romualdo Lagoon 3 orders of magnitude faster than that recorded from any modern region

of phosphogenesis. It is therefore unlikely that dissolved phosphorus diffusing up from the sediment was precipitated *directly* in the soft tissues of the fish. Indeed, Martill (1988, p12) himself stated that "It is difficult to see how so much phosphate can be rapidly dumped unless *vast* quantities of phosphate-enriched pore water are flushed through the system" (my italics).

b) Physiochemical cycling of the redox pair Fe^{3+}/Fe^{2+}: Berner (1973) and Benmore *et al.* (1983) have suggested that the level of dissolved phosphorus in anoxic sediments may be subsidised by the reduction of hydrous ferric oxides and hydroxides onto which phosphate ions were adsorbed in the oxygenated upper water column. Phosphate adsorbed to the surface of clay particles is similarly affected at redox boundaries (Moshiri and Crumpton, 1978). This mechanism has been invoked as the cause of the high levels of dissolved phosphorus in several lagerstätten (e.g. Allison, 1988a; Cater *et al.*, 1989).

Palaeoenvironmental and geochemical evidence (see Section 2.5.4) strongly suggests the upper few centimetres of sediment in the Romualdo Lagoon to have been dysoxic for the majority of time. The abundance of pyrite and absence of burrowing implies the sediment to have been anoxic only a short depth beneath this layer. Adsorbed phosphate ions would therefore have been released just beneath the sediment/water interface, and thus probably provided an important flux of phosphorus to the interstitial waters of the Romualdo Lagoon (see text fig. 7.1).

7.2.2 CONCENTRATING THE DISSOLVED PHOSPHORUS (refer to text fig. 7.1).

Although organically-bound and particulate-adsorbed phosphorus were almost certainly the immediate sources of phosphorus for the phosphatization of soft tissues in fish from the Romualdo Member (see Section 7.2.1), they (even in combination) were probably not released in sufficient quantities or fast enough to account for the quantity of phosphatized soft tissues in some carcasses (assuming fluxes of phosphorus from the sediment in to the overlying water column of the Romualdo Lagoon to have been comparable to those of modern regions of phosphogenesis, see calculation 7.4). Sufficient phosphorus may only have been released if it had first been concentrated in the sediment over a period of time prior

to release. Indeed, without a mechanism of concentrating the phosphorus released from the sediment, it would simply have diffused back up into the overlying water column (Filipek and Owen, 1981; Elderfield *et al.*, 1981; Suess, 1981; Froelich *et al.*, 1982). Allison (1988a) has also suggested that without a mechanism of concentrating dissolved phosphorus in the sediment, calcite precipitation will be favoured over apatite due to the enormous quantities of HCO_3^- which is liberated simultaneously with phosphorus during anaerobic microbial respiration.

I propose two mechanisms were involved in preventing the dissolved phosphorus from escaping from the sediment into the water column of the Romualdo Lagoon. These are:

1) Adsorption to clays and ferric hydroxides: Dissolved phosphorus diffusing up from the sediment of the Romualdo Lagoon may have been prevented from escaping into the water column by becoming adsorbed onto suspended clays and ferric hydroxides in the sediment's dysoxic upper zone (see Section 2.5.4). Although this zone was probably not particularly thick (a few tens of centimetres), and the volume of particulate material in it was limited (the sediment surface had a porosity of ≈85%, see Section 2.5), clays and ferric hydroxides have a high specific surface area and are therefore capable of adsorbing vast quantities of phosphorus. This layer of sediment would therefore probably have acted as a very effective barrier against the loss of phosphorus from the sediment (see text fig. 7.1). In contrast, bicarbonate ions would have been free to diffuse into the water column across the oxygenated zone, and would also have been continually removed from the system through the precipitation of the abundant and large $CaCO_3$ concretions (see Section 2.5). An oxygenated zone of sediment has been invoked as a mechanism of concentrating phosphorus in the sediment of a number of other systems (see Moshiri and Crumpton, 1978; Allison, 1988a; Cater *et al.*, 1989, p14).

2) Binding to algal mats: Phosphorus released from organics in the sedimentary pile may also have been prevented from escaping the sediment by being temporarily fixed in the microbial mats which draped over the sediment's surface (see Section 2.5). Phosphorus released by the death and decay of these mats, would become adsorbed onto clays and ferric hydroxides (see text fig. 7.1). In such a way, the phosphorus would have been continually recycled but maintained in the sediment (where phosphatization took place). A somewhat

similar role has been invoked for microbial mats in the development of primary phosphorites (Soudry, 1992; Reimers *et al.*, in press).

These two mechanisms would have dramatically increased the concentration of phosphorus in the upper few centimetres of sediment in the Romualdo Lagoon, and are consistent with bedding-plane parallel fish (i.e. those that did not sink excessively into the substrate) containing greater quantities of phosphatized soft tissues than transgressive fish (i.e. those that sunk below the sediment surface) (see Section 4.2.1). Similarly, Veeh *et al.* (1973) and Manheim *et al.* (1975) have recorded the greatest developments of apatite in active regions of phosphogenesis to be concentrated just above the oxygen minimum zone of the sediment. This implies a common mechanism of concentrating the phosphorus with regions of active phosphogenesis.

7.2.3 RELEASE OF THE PHOSPHORUS (refer to text fig. 7.1).

Krom and Berner (1981) have demonstrated phosphorus adsorbed to oxygenated clays and ferric hydroxides to be released almost immediately by anoxia (within 1hr). In the Romualdo Lagoon, phosphorus adsorbed to clays and ferric hydroxides in the upper few centimetres of the sediment may have been released to the interstitial fluids by the anoxic decay-halo of decaying fish (see text fig. 7.1). Taphonomic experiments indicate that at only 20 hours postmortem (at 20^0C, 3.5% salinity) and prior to any obvious signs of decay, fish carcasses can establish a large decay-halo (see text figs. 8.4 and 8.8). The concentration of dissolved phosphorus in the interstitial fluids of the sediment surrounding carcasses in the Romualdo Lagoon may have been further enriched by the release of phosphorus from the microbial mats. This requires the microbial mats to die. This too could have been triggered by the anoxic conditions created around the decaying fish, and/or by the release of toxins from the decaying carcasses.

Accepting phosphorus to have been released from the sediment by the incipient decay of the very organisms being phosphatized, it is conceivable that it was released either: a) *en mass* from the entire sediment by the decay of millions of closely spaced fish killed in a mass mortality event, or b) selectively around an individual fish. These two models are discussed separately below:

a) *En mass* release of phosphorus: Mass mortalities of fish may induce widespread reducing conditions at the sediment/water interface (Allison and Briggs, 1991a, p29). In the Romualdo Lagoon, the simultaneous death and decay of millions of fish killed in mass mortality events (see Section 2.5.5) would probably have resulted in the reduction of the sediment and the release of any adsorbed phosphorus *en mass* into the interstitial waters. Such events would completely deplete the phosphorus in the sediment pile, and thus, a supply would not be available to subsequent mass mortalities for some time later. This provides an explanation for the presence of phosphatized soft tissues in some, but not all fish from the same locality.

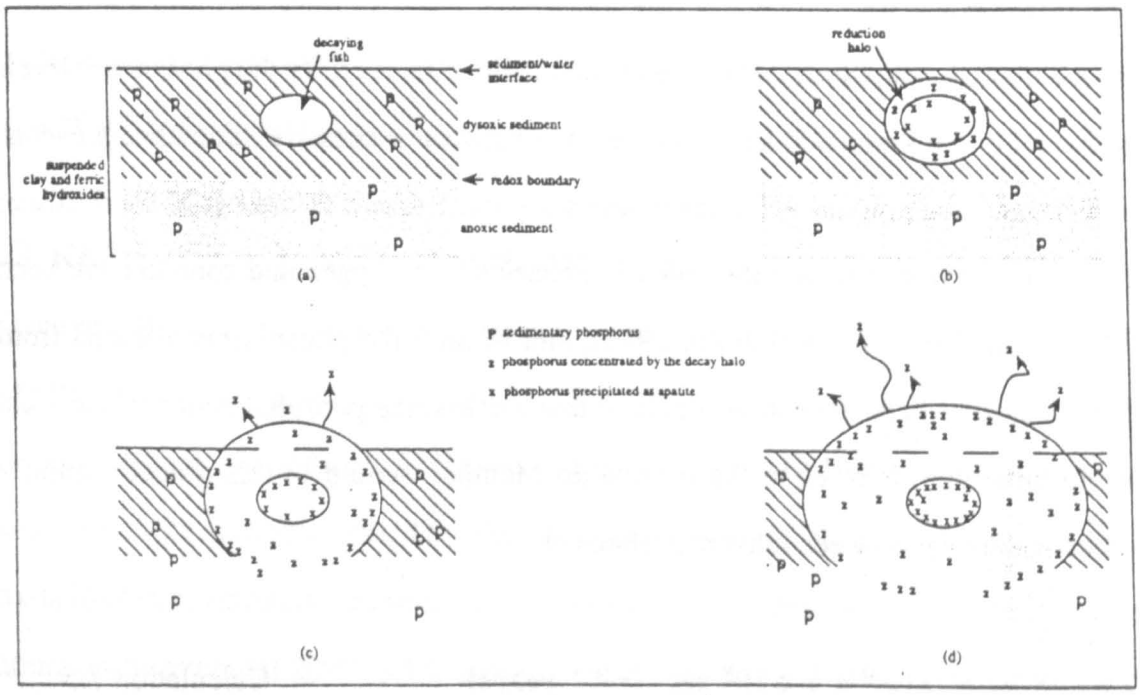
Making certain assumptions, one can estimate the time required for the phosphorus levels in the sediment of the Romualdo Lagoon to recover between successive mineralizing events. Making the same assumptions as for calculations 7.1 and 7.4, and assuming: a) the diffusive flux of phosphorus from beneath the redox boundary to the oxygenated zone to have been 200 $x10^{-9}\mu Mcm^{-2}sec^{-1}$ (Burnett *et al.*, 1982), and b) only the phosphorus released from beneath each carcass to have been available to that carcass; the phosphatization of the most extensively mineralized fishes in the Romualdo Member, would in this model require a period of concentration between mass mortalities of:

$(12258/200)/200\ x10^{-9}$ **= 3 x 10^8 secs (=9.7 years)** Calculation 7.5

This model of course assumes that all of the phosphorus released from the sediment entered the fish carcasses. This is somewhat unrealistic, but can be off set against the extra phosphorus which may have been available (but which I do not account for in calculation 7.5) from the carcasses of fish killed in earlier mass mortalities.

b) Selective release of phosphorus: In this model, the decay of an individual fish (which if forming part of a mass mortality is separated from its neighbour by some distance ≈1m) creates a localized area of reduction within which adsorbed phosphorus is released to solution. In contrast to the first model (see above), the continued occurrence of dysoxic conditions beyond the fish's decay-halo prevents any phosphorus liberated from the sediment from diffusing into the water column (text fig. 7.2b). Instead, as the diameter of

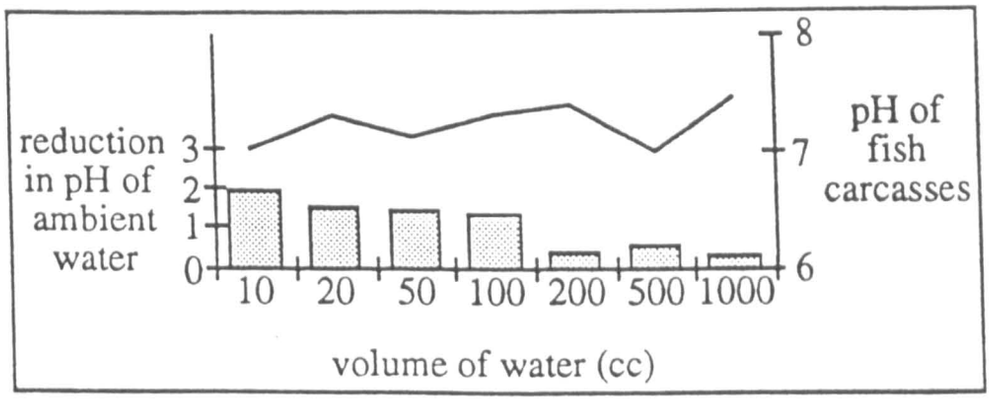
the decay-halo increases, the phosphorus must either diffuse into the carcass or become re-adsorbed onto peripherally located ferric hydroxides. This re-adsorbed phosphorus will again be released to the interstitial fluids as the decay-halo grows further (text fig. 7.2c and d). This 'recycling' process ensures that the majority of phosphorus adsorbed to the sediment affected by the final diameter of the decay-halo does not escape the vicinity of the fish, and is therefore always available to its soft tissues.



Text figure 7.2: Selective release of phosphorus adsorbed to ferric hydroxides in a soupy sediment by the growth of a reducing halo around a decomposing fish. a) A fish enters the dysoxic zone of a soupy substrate with a high concentration of adsorbed phosphorus. b) Decay of the fish creates a reducing halo which releases phosphorus from the sediment. c and d) Further decay results in a larger volume of sediment being reduced and the phosphorus either precipitated in the carcass, and/or concentrated at the periphery of the decay halo.

Actualistic taphonomic experiments indicate that decaying fish are capable of influencing the chemical environment (e.g. pH, level of oxygen etc) of extremely large volumes of water (relative to their own volume) even after relatively short periods of decomposition. An inverse relationship exists between the volume of water available to a decomposing fish and the extent to which that water's chemistry is altered. That is, the smaller the volume of water, the greater the change in its chemistry. For example, at 55hrs postmortem (20^0C),

whitebait (≈2.5cm^3) may affect the pH of up to 1 litre of water (or 400 times their own volume) although large shifts in pH occur only in volumes of <100ml (text fig. 7.3). Thus, in the soupy sediment of the Romualdo Lagoon where porosities were >85% (see Section 2.5), a 25cm fish could be expected to have greatly influenced the Eh and pH of a volume of sediment in excess of 40 litres.



Text figure 7.3: The effect of decaying whitebait (≈2.5cm^3) at 55 hrs postmortem on the pH of various volumes of seawater (20^{0}C). The pH of the fish themselves at 55 hrs postmortem is not affected by the volume of water in which decay took place.

Because of the large volume of sediment which may be reduced by a single fish, and the progressive increase in the concentration of phosphorus in the decay halo of decaying fish with time (see above), phosphatization according to this model may take place in sediments with a lower concentration of phosphorus than that invoked for the previous model (or after the sediment has had a shorter period of recovery). Assuming: a) the diffusive flux from beneath the redox boundary to the oxygenated zone in the Romualdo Lagoon to have been 200 x10^{-9} μMcm^{-2}sec^{-1}, and b) 25cm long *Notelops* and *Rhacolepis* to have reduced 1500cm^2 of dysoxic sediment immediately surrounding them in 55hrs; the most heavily mineralized fish (PRW/11) would require phosphorus to be concentrated in the sediment (prior to the death of the fish) over a period of:

$12258/(1500x200\ x10^{-9})$ = **40.8 x 108secs (=1yr 3months)** Calculation 7.6

DISCUSSION: Both of the above models have a number of encouraging features. In particular, they account for the mobilization of phosphate ions from the sediment at speeds

compatible with the timing of mineralization that I have estimated for the fish (see Chapter 6). The restriction of phosphatization only to dysoxic sediments provides both an explanation for the non-mineralization of soft tissues at certain times and/or regions of the Romualdo Lagoon (i.e. those that were anoxic, see Section 2.5.4), and for the selective phosphatization of soft tissues only in bedding-plane parallel fish (i.e. those that were in the upper oxygenated zone). Furthermore, the adsorption of phosphorus to suspended particles removes problems experienced by Martill's (1988, 1989b) models concerning both the stabilization of solutions supersaturated with respect to apatite, and the dilution of microbially-released phosphorus in the vast volume of the hypolimnion.

7.3 THE SOURCE(S) OF PHOSPHORUS IN OTHER LAGERSTÄTTEN

External sources: An identical source to that in the Romualdo Member (see Section 7.2.1) cannot be assumed for all occurrences of phosphatized soft tissues. Nevertheless, shared palaeoenvironmental characteristics and preservational styles with fish from the Romualdo Member suggests the phosphorus in a number of other deposits to have had a similar provenance. For example, the exceptional state of preservation of phosphatized soft tissues in fish from the Cordillera de Domeyko (see Section 5.2.6), the Glencartholm Volcanic Beds (see Section 5.2.13), and the Cleveland Shale (see Section 5.2.14) makes it difficult to envisage decay as having advanced to the point at which phosphorus could have been released from the fossilized tissues themselves. Indeed, with the exception of microbes on the outer surface of some fish muscle from the Glencartholm Volcanic Beds (see fig. 5.19), micro-organisms (which are essential for the release of organically-bound phosphorus, see Section 7.2.1) are extremely rare in these tissues. Taphonomic experiments indicate that other organs in these fish were also unlikely to have decomposed to a sufficient extent at the time of mineralization to have provided phosphorus to the phosphatized soft tissues. Therefore, the phosphorus in these deposits would appear to have been derived predominantly from an external source. As in fish from the Romualdo Member however, the frequent preservation of the digestive tracts of fish from both the Cordillera de Domeyko (Schultze, 1989) and the Cleveland Shale (Dean, 1902, 1909) suggests that the stomach

contents of these fish may have supplied *some* of the phosphorus for the phosphatization of their alimentary tracts.

An external source of phosphorus was probably also involved in the phosphatization of soft tissues in the ostracodes (Bate, 1971, 1972, 1973), copepods (Cressey and Patterson, 1973; Cressey and Boxshall, 1989), and anostracans (Maisey, 1991, p410) in the Romualdo Member. The soft tissues of these organisms are only preserved when associated with large vertebrate fossils. Bate (1972) has argued that this (at least for the ostracodes) indicates the phosphorus to have been derived from the associated decomposing vertebrates. However, one may instead argue that the soft tissues of these micro-invertebrates are only preserved (or at least have only been recorded) when associated with large carcasses, because independently, they were incapable of reducing a sufficient volume of sediment to release enough phosphorus for their phosphatization.

Undoubtedly however, associated masses of organic material were responsible for the selective phosphatization of some micro-invertebrates (as in Bate's, 1971 model). The most obvious examples involve the association of micro-invertebrates composed of recalcitrant biomolecules (e.g. the chitin of arthropods) with large fossils in deposits which do not normally yield phosphatized soft tissues. The rapid decay of the large carcass relative to the stable tissues of the associated micro-invertebrate ensures that the latter is provided with a concentrated and sustained source of phosphorus for the mineralization of its soft tissues. Likely examples include an ostracode from the stomach of an Upper Jurassic ichthyosaur in the USSR (Dzik, 1978), and an ostracode from a Wealden coprolite (Bertrand, 1903). Part of the phosphorus required for the mineralization of soft tissues in shrimps in the stomachs of fish from the Romualdo Member (Wilby and Martill, 1992) may also have been derived from the fishes. Another, although as yet unconfirmed example, is the phosphatized gills of inoceramids from the Cretaceous Niobrara Chalk Formation, Kansas (Stewart, 1990). Here, the decay of hundreds of enclosed commensal fish *may* have provided the necessary flux of phosphorus for the mineralization of the bivalve's soft tissues.

Internal sources: In several lagerstätten, the phosphorus appears to have been derived from the organisms actually containing the phosphatized soft tissues (i.e. the source is

internal). Briggs and Kear (1993a) have experimentally achieved such mineralization in the lab. In their experiments, the musculature of shrimps was phosphatized by phosphorus released by bacteria from the shrimp's cuticles. Briggs and Kear's (1993a) experiments provide a convincing analogy for a number of deposits in which phosphatized soft tissues are restricted entirely to a single faunal element which contains a high concentration of organically-bound phosphorus (most typically arthropods). The absence of phosphatized soft tissues in other organisms in these deposits negates an external source of phosphorus. Examples include the shrimp - *Tealliocaris* - from the Gullane shrimp bed (see Section 5.2.11); ostracodes from the Upper Devonian Cephalopod Limestone of the Carnic Alps (Müller, 1982), and ostracodes and other invertebrates from the Lower Cambrian Comley and Rushton Fauna of Shropshire (Hinz, 1987).

Briggs and Kear's (1993a) experimental results may also be extrapolated to the situation in the Portland Roach where despite the presence of a relatively diverse molluscan fauna, phosphatized soft tissues appear to be restricted entirely to two species of trigoniids (see Section 5.2.4). An external source of phosphorus cannot therefore be assumed. Since the soft tissues of the trigoniids are preserved only by a coating of microbes, it is unlikely that the infesting microbes had metabolized sufficient quantities of the tissue's organically-bound phosphorus to have caused their own mineralization. Sufficient organically-bound phosphorus would probably only have been released if the fossilized soft tissues had been thoroughly decomposed and completely infested by microbes. Instead, it would appear that the source of phosphorus in this case was those organs in the bivalves that are not preserved; their decay providing sufficient phosphorus for the mineralization of those tissues which were only partially decomposed. The involvement of this source in the phosphatization of these soft tissues is supported by a number of lines of evidence. These are: 1) My taphonomic experiments indicate that the mantle (the tissue most commonly preserved in the trigoniids) of *Mytilus* sp. is consistently the most decay-resistant tissue in bivalves, and would therefore probably have been intact in the trigoniids even after all of their other soft tissues had decomposed. 2) Phosphatized soft tissues occur only in articulated and tightly closed specimens of the trigoniids. This would have guaranteed that all of the phosphorus released from their soft tissues was retained within the bivalves. 3) Mass balance

calculations based on the concentration of phosphorus in the soft tissues of Recent bivalves (Vinogradov, 1953) suggest that the bodies of *L. gibbosa* and *M. incurva* did contain sufficient phosphorus (assuming all was released by the microbes) to account for the mass of apatite precipitated in their mantles. Indeed, Hirschler (1990) has succeeded in phosphatizing the soft tissues of *Mytilus* sp. when the tissues themselves were the only source of phosphorus.

In several other lagerstätten too, an internal source of phosphorus is most compatible with the fossil evidence. For example, phosphatized soft tissues in both Eckfeld Maarlake (see Section 5.2.1) and Lake Odernheim (see Section 5.2.10) are extremely rare, restricted to a single taxon, and are entirely structureless and heavily infested with microbes. This is consistent with an internal source of phosphorus. Indeed, Micklich and Wuttke (1988) and Willems and Wuttke (1987) respectively have both suggested that all of the phosphorus for the mineralization of these tissues was derived directly from the decomposing organisms themselves.

Only after a detailed investigation will it be possible to establish more confidently the provenance of phosphorus in many of the other deposits discussed in Chapter 5. Briggs and Kear (1993a) have suggested "closed" anoxic conditions to be one of the most important requisites for the phosphatization of the soft tissues when the source of phosphorus is internal. Such conditions must have occurred in abundance in the fossil record, particularly in cases where organisms were buried catastrophically in anoxic sediments (see Brett and Seilacher, 1991). Phosphatized soft tissues are not correspondingly abundant. Therefore, in the majority of occurrences of phosphatized soft tissues (particularly in deposits in which phosphatization was a recurring phenomenon), it is my opinion that at least some (if not all) of the phosphorus was derived from an external source.

There is however much work remaining to be done regarding the source(s) of phosphorus. This would be greatly facilitated by more experimental attempts at phosphatizing soft tissues in the lab. (e.g Briggs and Kear, 1993a; Briggs *et al.*, 1993). These would permit phosphate sources suspected in the fossil deposits to be tested directly.

Deposits (given in stratigraphical order) Those in which phosphatization was dominated by an external (sedimentary) source of phosphorus are given in the top of the table	Salinity					Lithology				Burial rate			Oxygen levels at sed./water interface			Basin		
	Hyper-saline	Normal marine	Brackish	Fresh-water	Non-marine Hyper-saline	Limestone	Mudstone	Limestones in Mudstones/ Silts	Other	High/ Catastrophic	Moderate	Low	Aerobic	Dysaerobic	Anaerobic	Enclosed	Restricted	Open
Hagel Basin		×				×				×						×		
Romualdo Member			–					×			×			–			×	
Crato Formation	×					×					×					×		
Solnhofen Limestone	×					×				×							×	
Cordillera de Domeyko		×						×			?				–			×
Oxford Clay Formation		×					×				×							×
Lombardische Kieselkalk Formation		×				×					?					×		
Lower Lias		×						×				×						×
Granton Shrimp Bed								×				?					×	
Glencartholm Volcanic Beds								×			?						×	
Bearsden								×			?		?		?		?	
Cleveland Shale								×			?						×	
Alum Shale Formation		×						×			×							×
Eckfeld maarlake				×			?					?				×		
Niobrara Chalk Formation		×				×												×
Portland Roach		×				×				×								×
Lake Odernheim					×			×			?					×		
Gulliane Shrimp Bed								×				×					×	

Table 7.1: Palaeoenvironmental characteristics of deposits yielding phosphatized soft tissues

7.4 A COMMON PROVENANCE OF IONS? (refer to table 7.1)

An external source of phosphorus would appear to be one of the most important prerequisites (but certainly not the only, see Section 8.2) for soft tissue phosphatization. It would therefore be of considerable interest, particularly from the point of view of prospecting, to identify palaeoenvironmental characteristics shared by all cases of postmortem phosphatization where an external source is suspected.

Phosphatized soft tissues appear to have occurred randomly through time from the Cambrian (e.g. the Alum Shale Formation, see Section 5.2.15) to the Recent (e.g. the Potterne midden, see Piearce *et al.*, 1990). There does not appear to be any correlation between the occurrence of phosphatized soft tissues and global periods of phosphogenesis (see Cook and McElhinny, 1979, pp323-327), secular variations in the mineralogy and geochemistry of marine carbonates (see Tucker, 1992), or global sea level curves (see Wilgus *et al.*, 1988). Indeed, since many deposits containing phosphatized soft tissues are non-marine or not fully marine (see Chapter 5) it would be surprising to identify such correlations.

Phosphatized soft tissues mineralized by an external source of phosphorus would therefore appear to be specific to a certain combination of exceptional environmental conditions (see below). In contrast, organisms which I consider to have been phosphatized by an internal source of phosphorus, or by phosphorus derived from a large associated mass of decaying organics (see those deposits confined to the bottom of table 7.1), do not appear to be characteristic of any particular palaeoenvironment (see table 7.1), although depositional settings prone to catastrophic burial or the growth of algal mats may be favourable due to the removal of the carcasses from scavengers (Brett and Seilacher, 1991), and the promotion of anoxic "closed" conditions (Briggs and Kear, 1993a). Since a source of phosphorus is already available, these soft tissues do not have to rely on the occurrence of environmental conditions favourable to the concentration of phosphorus (as do soft tissues phosphatized by an external source of phosphorus). My comments below are therefore restricted only to soft tissues phosphatized by an external source of phosphorus.

The particular conditions required for the phosphatization of soft tissues were repeatedly met in the Lower Carboniferous coastal delta-plain sediments of northern Britain (see Cater

et al., 1989). Cater *et al.* (1989, p14) considered 5 variables to have been particularly important in promoting the phosphatization of soft tissues in these sediments. These are: 1) the quantity and timing of organic input; 2) the concentration of dissolved phosphorus; 3) the rate of sedimentation; 4) the position of the redox boundary; and, 5) the salinity. The position of a number of the lagerstätten discussed in Chapter 5 relative to these parameters and the 'degree of basin enclosure' are given in table 7.1. The importance of each of these parameters to the phosphatization of soft tissues is discussed individually below:.

1) SALINITY: Experimental evidence suggests apatite precipitation to be favoured by hypersaline conditions (Nathan and Sass, 1981). However, according to table 7.1, occurrences of phosphatized soft tissues are not salinity-controlled. The greatest number of occurrences phosphatized soft tissues are in normal marine and brackish water depositional settings. This however, may simply be a consequence of the relative frequency with which these environments are preserved in the stratigraphical record.

Salinity may however play an indirect role in the phosphatization of soft tissues. Under adverse salinity conditions (i.e. very high salinities or brackish salinities), benthic organisms will be restricted in diversity and abundance. This will greatly diminish (relative to a hospitable system) the quantity of phosphorus recycled by infaunal detritus feeders and made unavailable (because of its precipitation as inert skeletal elements) to precipitate in soft tissues.

2) LITHOLOGY: All occurrences of phosphatized soft tissues that I interpret to have had an external source of phosphorus, occur in organic-rich sediments (e.g. mudstones and plattenkalks)(see table 7.1). In agreement with Allison (1988a, pp338-339), Martill (1988), Cater *et al.* (1989, p14), and my model for the Romualdo Member (see Section 7.2.1), this suggests that disseminated organic material provides the most important external source of phosphorus for the phosphatization of soft tissues. It is also obvious from table 7.1 that carbonate concretions are an important requisite for the preservation of phosphatized soft tissues. The relevance of early diagenetic concretions to the preservation of three-dimensional fossils has been emphasized by both Müller (1985) and Martill (1988).

However, their participation in the removal of HCO_3^- and therefore their role in stimulating the phosphatization of soft tissues rather than their calcification has not previously been recognised. It is likely that the carbonate fraction of plattenkalks plays a similar role.

3) RATE OF SEDIMENTATION: Allison (1988a, pp338-339) has argued that phosphatization of soft tissues will be favoured by a low rate of burial, since this increases "the residence time at the anoxic/oxic interface, where phosphate concentrations may be secondarily enriched by the reduction of ferric iron". This apparently agrees with Van Cappellen and Berner's (1988, p311) investigations which indicate that the greatest fluxes of phosphorus occur in sediments with the slowest rates of burial. However, I suggest that sedimentation rates have little control over the processes of soft tissues phosphatization. This is because:

a) ferric hydroxides and clays react extremely rapidly to fluctuations in Eh (Krom and Berner, 1981). Therefore, relative to even high rates of burial, phosphorus will almost instantaneously 'relocate' itself in the sediment according to the position of the redox boundary.

b) rates of phosphate diffusion in sediments are rapid (Suess, 1981; Burnett *et al.*, 1982) even relative to high rates of sedimentation (excluding catastrophic burials). Therefore, even after a period of intense sedimentation, levels of dissolved phosphorus will rapidly re-establish themselves in the surface layers of the sediment by the diffusion of phosphorus up from the previous sediment surface.

c) negligible quantities of sediment would have been deposited over the period of time in which phosphatization took place (≈55hrs, see Chapter 6), and therefore the position of the redox boundary is unlikely to shift.

The unimportance of sedimentation rate on the likelihood of soft tissues being phosphatized is supported by the enormous variation in the rates of deposition displayed by lagerstätten containing phosphatized soft tissues (table 7.1).

4) OXYGEN LEVELS AT THE SEDIMENT/WATER INTERFACE: Phosphatization appears to be restricted to sediments with anoxic or dysoxic background conditions. Assuming the phosphorus in all cases to have been derived from organics in the sediment (see above), this begs the question as to how the phosphorus was prevented from escaping the sediment and thus made available to invade soft tissues. It is clear from the fluxes of dissolved phosphorus in Recent sediments (Suess, 1981; Burnett *et al.*, 1982) that a mechanism of concentrating the phosphorus released from the sediment pile is required if tissues as volatile as muscle are to be phosphatized (see calculation 7.4). Without such a mechanism, most soft tissues would decay before enough phosphorus diffused into them.

In those sediments which experienced even limited oxygenation, it is likely that clays and ferric hydroxides acted as a phosphorus trap in a similar manner to those in the Romualdo Lagoon (see Section 7.2.2). Indeed, in some of the deposits (e.g. the Oxford Clay Formation [Duff, 1975] and the Cleveland Shale [Dean, 1902]), the sediment is known to have had an oxygenated upper suspended layer. In those lagerstätten which are recorded as being anoxic in table 7.1, it is possible that phosphatization coincided with short-lived periods of oxygenation. Circulation of the bottom waters has been proposed, or may be inferred, from a number of such deposits including the Haqel basin (Hückel, 1970) and the Solnhofen Limestone (Seilacher, 1963). Since phosphatized soft tissues in these deposits appear to be relatively rare, it would not be necessary to invoke prolonged or frequent periods of circulation, but merely occasional and short-lived episodes.

In other deposits, microbial mats may have played a more dominant role in concentrating phosphorus in the sediment. These have been recorded from the Crato Formation (see Section 2.4.4), the Solnhofen Limestone (see Seilacher *et al.*, 1985, pp14-15), the Granton shrimp bed (Briggs and Clarkson, 1983, p162), and the Glencartholm Volcanic Beds (Cater *et al.*, 1989, p8).

In those deposits for which oxygenated bottom waters and microbial mats cannot be inferred (e.g. the Lombardische Kieselkalk Formation), one must conclude that either a) the sediment surface experienced periods of oxygenation too short-lived to be identified by palaeoenvironmental evidence; b) the sediment was capable of producing a greater flux of phosphorus than is recorded for contemporary regions of phosphogenesis; c) an alternative

mechanism of concentrating dissolved phosphorus was involved; and/or, d) the phosphorus was actually derived from an internal source.

It is interesting to note that in both of the deposits listed in table 7.1 which experienced prolonged periods of oxygenation - the Portland Roach and the Niobrara Chalk Formation - the phosphorus was derived from an internal source, and the phosphatized soft tissues occur only in those organisms which were capable of maintaining a reducing microenvironment completely isolated from the external environment (i.e. within the tightly closed valves of bivalves). This suggests that the concentration of dissolved phosphorus in well oxygenated sediments is not as great as that in anoxic ones. This assumption is consistent with recorded differences in the concentration of phosphorus in the interstitial waters of aerobic and anoxic sediments (Sholkovitz, 1973). Sholkovitz (1973, p2068) proposed these differences to be related to the limited availability of reactive organic matter in oxygenated sediments relative to anoxic sediments (following aerobic decay at the sediment/water interface), and the subsequent effect this has on the extent of sulphate reduction. The quantity of phosphorus immediately available to soft tissues in oxygenated sediments may also be reduced (relative to dysoxic and anoxic sediments) by its temporary (or permanent) removal by infaunal detritus feeders (see above).

5) BASIN ENCLOSURE: Stagnant basins have been proposed as major areas of phosphorite deposition by some authors (e.g. Brooks *et al.*, 1968). In comparison to the relative rarity of enclosed and restricted environments in the stratigraphical column, such environments are well represented in table 7.1. This suggests a causative relationship between the phosphatization of soft tissues and the degree of basin enclosure. This may correspond to the propensity of restricted depositional environments to: 1) be highly productive and to stimulate the preservation of sediments with a high organic carbon content, 2) have poorly oxygenated sediment surfaces, and 3) have stratified water columns with hypersaline hypolimnions. Although more frequently preserved in the stratigraphical column, phosphatized soft tissues are comparatively rare in 'normal', open marine sediments.

Müller (1985, pp72-73) has listed a number of sedimentological features which he has utilized to improve his success with prospecting. Among these he suggests still-water conditions and nodular limestones with a high phosphatic content to be particularly important. In addition to these, I suggest 4 other palaeoenvironmental characteristics are distinctive of deposits containing phosphatized soft tissues. These are: 1) a high organic content (Allison, 1988a), 2) deposition within an enclosed basin, 3) an oxygenated sediment/water interface, and 4) a restricted or non-existant benthic fauna.

7.5 CONCLUSIONS

1) For kinetic reasons, apatite is incapable of precipitating in soft tissues directly from the water column; phosphorus must first be concentrated in a microenvironment either organically or inorganically.

2) Soft tissues may be phosphatized by either an internal, and/or external source of phosphorus. Organisms phosphatized *wholly* by an internal source of phosphorus are rare; in most cases, an external source is necessary to create a sufficient saturation of apatite. The most important internal sources of phosphorus are the organism's gastric contents, and the microbially-mediated decay of the organism's own soft tissues. The most important external source of phosphorus is that released by microbes from disseminated organics in the sediment.

3) Phosphatized soft tissues are restricted to those fish in the Romualdo Member that were located in the upper few centimetres of the dysoxic soupy sediment. Gradients in the density of mineralization of soft tissues in the Romualdo Member fish indicate the *dominant* source of phosphorus to have been external to the carcasses. This was probably derived from sedimentary organic detritus.

4) Phosphatization of soft tissues in fish from the Romualdo Member was spasmodic (i.e. not all fish from a single locality contain fossilized soft tissues). Phosphate fluxes in Recent sediments suggest that phosphatization in the Romualdo Lagoon could only occur if a mechanism of concentrating the phosphorus released from the organic-rich sediment existed. Phosphorus was probably concentrated in the upper few centimetres of the sediment by

adsorption onto clays and ferric hydroxides, and by being temporarily organically-bound in microbial mats.

5) Phosphorus concentrated at the sediment/water interface of the Romualdo Lagoon was released by the decay of large organisms. The reduction in Eh and release of toxins associated with their decay, reduced the ferric hydroxides and killed the microbial mats respectively, thereby releasing phosphorus to the sediment's interstitial fluids.

6) The presence of an external concentrated source of phosphorus is no guarantee that soft tissues will be phosphatized; phosphatization is controlled by a number of other variables (see Chapter 8).

7) Palaeoenvironmental characteristics shared by many lagerstätten discussed in Chapter 5 suggests that the source of phosphorus, and processes by which the phosphorus was concentrated in the sediment and then released to the sedimentary pore waters, were probably the same as in the Romualdo Lagoon.

8) Soft tissues fossilized by an *external* source of phosphorus are characteristic of specific sedimentary geochemistries and palaeoenvironments. Of particular importance is the early diagenetic growth of protective $CaCO_3$ concretions, a high concentration of sedimented volatile organics, deposition within a restricted environment, and a mechanism of preventing phosphorus from escaping the sediment. Palaeoenvironmental characteristics (in particular the sedimentology) shared by the Romualdo Member and regions of active phosphogenesis, suggest that the process of soft tissue phosphatization is merely an extravagant end-member of phosphogenesis.

9) Soft tissues mineralized by an internal source of phosphorus are not characteristic of any particular environment, although depositional settings prone to catastrophic burial or the growth of algal mats may be favourable due to the removal of the carcasses from scavengers, and the promotion of anoxic "closed" conditions.

CHAPTER 8
THE ROLE OF ORGANIC MATRICES IN THE INORGANIC PHOSPHATIZATION OF SOFT TISSUES

8.1 INTRODUCTION

The precision with which soft tissues are replicated by the process(es) of inorganic postmortem phosphatization has led a number of authors to interpret their fossilization in terms of traditional models of *in vivo* biomineralization (the mineralization of soft tissues in living organisms). Müller (1985, p70), Allison (1988d, p409), and Martill (1990a, p172) have suggested that specific biomolecules may act as "templates" for the precipitation of apatite in decomposing organisms, whilst Schultze (1989) even likened the process to pathological biomineralization in cattle.

Certainly, since phosphatization in all of the deposits described in Chapter 5 is restricted entirely to the organisms (none occurring as nodules or beds), the carcasses must have played some role in the precipitation of the apatite. The precision with which some tissues are replaced, and the repetitive preservation of the same tissues in a number of different organisms (see Chapter 5) suggests certain substrates did play a fundamental role in fossilization. However, since most traditional models of biomineralization require strict physiological and cellular regulation by a *living* system, they are incapable (without modification) of accounting for the fossilization of decomposing soft tissues.

This chapter examines the nature and extent of the participation of decomposing organisms and their constituent biomolecules in postmortem phosphatization. The ultimate goal is the development of a model which is capable of describing the mineralization of progressively degrading soft tissues. No attempt is made to describe the processes of microbial mineralization (for which the mechanisms of mineralization are relatively well understood, see Section 3.3.3); comments are restricted entirely to processes of *inorganic* phosphatization. Certain tissue/crystallite relationships in the fossil material has prompted me to propose a number of hypotheses which require testing. These are clearly signalled as such in the text.

8.2 DIFFERENTIAL PHOSPHATIZATION

Examination of phosphatized soft tissues from the Romualdo Member biota (see Chapters 4) indicates differential mineralization (or preferential phosphatization) to have occurred at three levels of organisation. These are: 1) the organism, 2) the tissue/organ, and 3) the subcellular/biomolecular level. Differential phosphatization in most cases cannot be attributed to any one cause; it would appear to be a complex interplay of a number of factors. Likely controls on differential phosphatization at each level are discussed separately below.

8.2.1 DIFFERENTIAL PHOSPHATIZATION OF ORGANISMS

Within the deposits examined in this study (see Chapter 5), differential phosphatization of organisms was expressed in one of three ways. These are by: a) the preferential phosphatization of soft tissues within specific groups of organisms, b) the preservation of soft tissues in one group of organisms but not in another closely related group, and c) by the preferential mineralization of soft tissues in certain organisms within a single deposit.

Even in those deposits which I consider to have had a large and reliable external source of phosphorus (e.g. the Romualdo Member, see Section 7.2), only a very small percentage of the fossils preserved actually contain mineralized soft tissues. The availability of phosphorus is therefore clearly not enough to guarantee the phosphatization of all soft tissues. A number of other conditions must first be satisfied. These are discussed below:

1) The concentration of phosphorus in the organism: According to Balson (1980), Müller (1985), and Allison (1988b), phosphate-rich organisms may act as nuclei for the precipitation of apatite, and therefore have a greater potential for phosphatization. Certainly a positive correlation does exist between the concentration of phosphorus in organisms and the frequency with which they contain phosphatized soft tissues. Although by no means the most commonly preserved fossils, fish, cephalopods, and crustaceans

	Cnidarians	Annelids	Brachiopods	unspecified	Arthropods: Trilobita	Arthropods: Crustacea	Arthropods: Chelicerata	Uniramia	Echinoderms	other	Chordata: Teleostomi	Chordata: Chondrichthyes	Chordata: Amphibians	Chordata: terrestrial reptiles	Chordata: Pterosaurs	Chordata: marine reptiles	Chordata: Birds	Mollusca: Gastropods	Mollusca: Bivalves	Mollusca: Cephalopods	Plants
Eckfeld maarlake (Section 5.2.1) ?											√										
Hagel Basin (Section 5.2.2) ?											√								×	×	
Romualdo Member (Chapters 2 and 4)		×				√			×		√	√		×	√			×	×		×
Crato Formation (Section 5.2.3)						×	×	√			√		×				×				×
Cretaceous USSR (Martinson *et al.*, 1986) ?																			√		
Volga River (Dzik, 1978) ?						√										×					
Portland Roach (Section 5.2.4)		×				×												×	√	×	×
Solnhofen Limestone (Section 5.2.5) ?	×	√				√	×	×	×		√	×		×	×	√	×		×	√	
Cordillera do Domeyko (Section 5.2.6)			×			×					√								×	×	×
Oxford Clay Formation (Section 5.2.7)			×			×					×	×				×		×	√	√	
Lombardische Kieselkalk Fm (Section 5.2.8)		√	×			√					√	√						×		√	×
Lower Lias (Section 5.2.9)			×			×		×	×		×	×		×		√			×	√	×
Sticky Keep Formation (Weitschat, 1983a, b) ?						√															
Saar-Nahe Basin (Section 5.2.10) ?													√								
Granton Shrimp Bed (Section 5.2.11)	√	√				√				√	×							×	×	×	√
Gullane Shrimp Bed (Section 5..2.12)	×					√	×				×										×
Glencartholm Volcanic Beds (Section 5.2.13)		×	×			×	√	×	×		×	√						×	×	×	×
Bearsden (Coates, 1993) ?											√										
Cephalopod Limestone (Müller, 1982b) ?						√														×	
Cleveland Shale (Section 5.2.14) ?			×							×		√									×
Lwr Ordovician, Sweden (Andres, 1989) ?				√																	
Alum Shale Formation (Section 5.2.15)				√	√	√															

Table 8.1: Taxonomic distribution of phosphatized soft tissues. ?=taxonomic data incomplete,√ =phosphatized soft tissues, X=organism present but not known to contain phosphatized soft tissues. Refer to the appropriate Section or reference for a more detailed discussion of each deposits sedimentology and palaeontology.

(which contain high concentrations of phosphorus, see Vinogradov, 1953) are the most commonly phosphatized organisms (table 8.1). However, contrary to Balson (1980), Müller (1985), and Allison (1988b), this correlation may not be a consequence of the propensity of these organisms for nucleating apatite (which has yet to be convincingly demonstrated). Instead, the preferential phosphatization of soft tissues in these organisms may be related to the greater quantity of phosphorus available to infesting microbes.

Since microbes are the most important mechanism by which organically-bound phosphorus is released (see Section 7.2.1), those organisms which have a propensity for microbial infestation (e.g. crustaceans, bivalves, and teuthids; see text fig. 5.6 and table 5.1) and also contain a high concentration of organically-bound phosphorus (e.g. crustaceans, See Vinogradov, 1953), will have a statistically greater chance of being phosphatized than those in which phosphorus is less abundant. This is most convincingly demonstrated by the relative frequency with which soft tissues are preserved in gastropods, cephalopods, and bivalves (see table 8.1). According to data given by Vinogradov (1953, tables 173, 174, 176), the 'wet' tissue concentration of phosphorus in gastropods (≈0.07wt%) is considerably less than that of bivalves (≈0.16wt%) and cephalopods (≈0.134wt%). Phosphatized soft tissues are correspondingly scarce (if not unknown) in gastropods. A similar conclusion was reached by Briggs and Kear (1993a).

All reports of phosphatized soft tissues in bivalves (with the exception of those from the Niobrara Chalk Formation, Stewart, 1990) are from taxa containing phosphatic gill supports (e.g. trigoniids from Kysylkum, USSR, Martinson *et al.*, 1986; and the Portland Roach, see Section 5.2.4; unionoids from the Lower Cretaceous, pers. comm. Whyte, Sheffield Uni.). There would therefore appear to be a causative relationship between the possession of mineralized gill supports and the postmortem phosphatization of their soft tissues. It is likely that the tissues and mantle cavity of these bivalves were buffered with phosphate during life as a precaution against the dissolution of their phosphatic elements. Upon death, these tissues would have been ideally preconditioned for the precipitation of apatite.

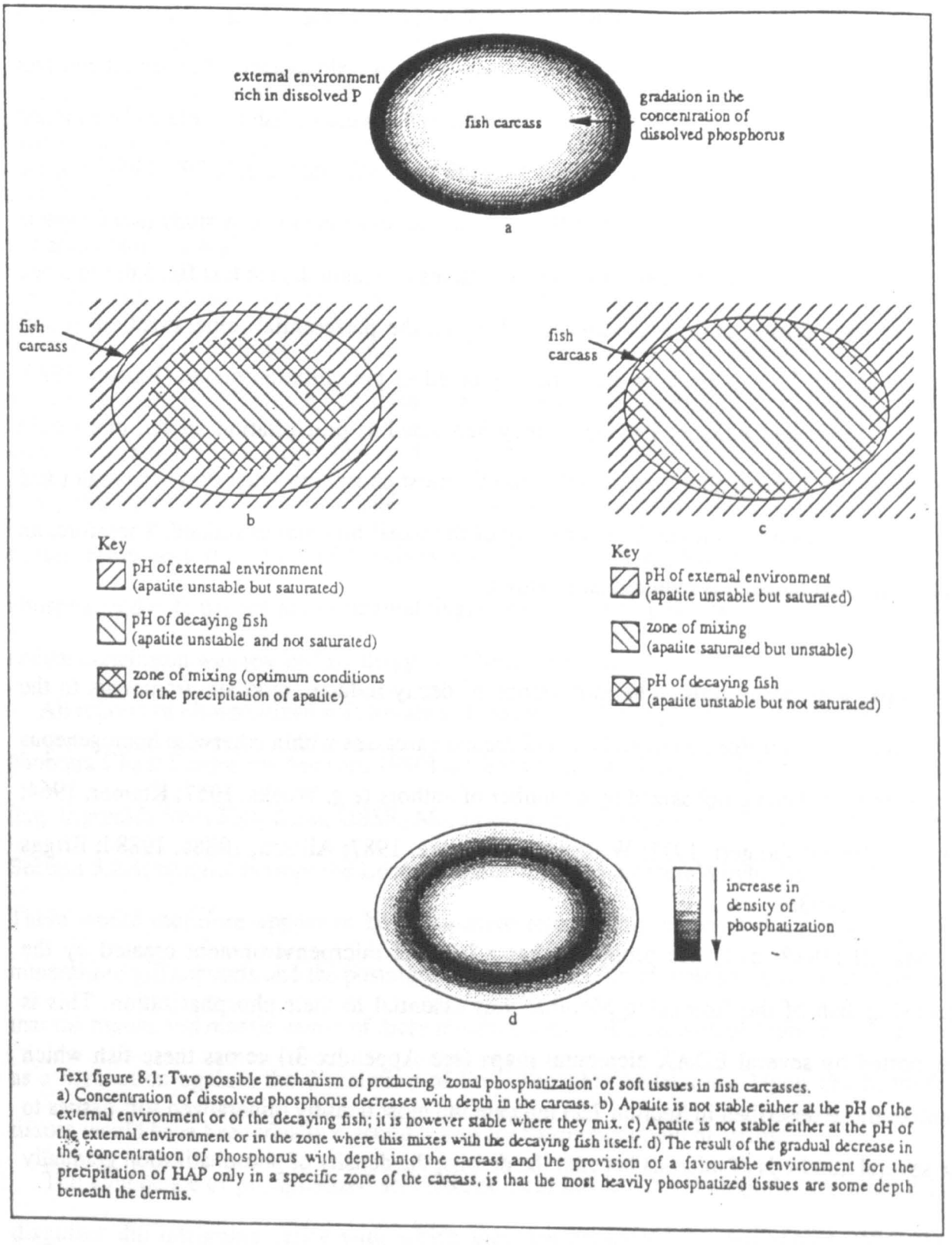
The abundance of phosphatized soft tissues recorded from cephalopods (see table 8.1) disguises the intriguing rarity with which they are preserved in ammonoids. With the exception of siphuncular membranes (e.g. see Weitschat, 1986; Pinna, 1985), phosphatized

soft tissues have not been recorded in ammonoids. In contrast, soft tissues are frequently preserved in teuthids (e.g. the Solnhofen Limestone, see Section 5.2.5; the Oxford Clay, Allison, 1988d; the Lower Lias, see Section 5.2.9). It is not clear whether differential mineralization within the Cephalopoda reflects differences in the concentration of phosphorus in their soft tissues, and/or an alternative factor (see below).

Certainly, differences in the abundance of phosphorus in the soft tissues of different fish taxa do not appear to be able to account for the relative abundance of phosphatized soft tissues in fossil fish (see table 8.1). Although the soft tissues of fish are relatively enriched in phosphorus (≈0.2wt%+ of wet tissue, Vinogradov, 1953, tables 290, 292, 295-297), the lack of infesting microbes in most of the fossil tissues examined in this study (see Chapters 4 and 5) indicates that this source (unlike in bivalves and teuthids, see text fig. 5.6) remained largely inert during phosphatization. Neither can the preferential mineralization of soft tissues in fish be explained by them having acted as precipitation nuclei (Balson, 1980; Müller, 1985; and Allison, 1988b). If they had acted as precipitation nuclei, one would expect apatite to have nucleated directly onto the most phosphorus-rich sites - the scales and bones. This has not been confirmed by any of the fossil material examined. Therefore, an alternative explanation is required (see below).

2) pH and decay halo: The importance of decay-induced microenvironments to the precipitation of authigenic minerals in and around carcasses within otherwise homogeneous sediments has been emphasized by a number of authors (e.g. Weeks, 1957; Kramer, 1964; Berner, 1968a; Zangerl, 1971; Willems and Wuttke, 1987; Allison, 1988c, 1988d; Briggs and Kear, 1993a).

Martill (1988, p11) has proposed that a low pH microenvironment created by the decaying fish of the Romualdo Member was essential to their phosphatization. This is supported by several EDAX elemental maps (see Appendix 3i) across these fish which (unlike those described in Section 7.2) indicate the most densely mineralized soft tissues to be several hundreds of microns beneath the dermis; the density of phosphatization gradually



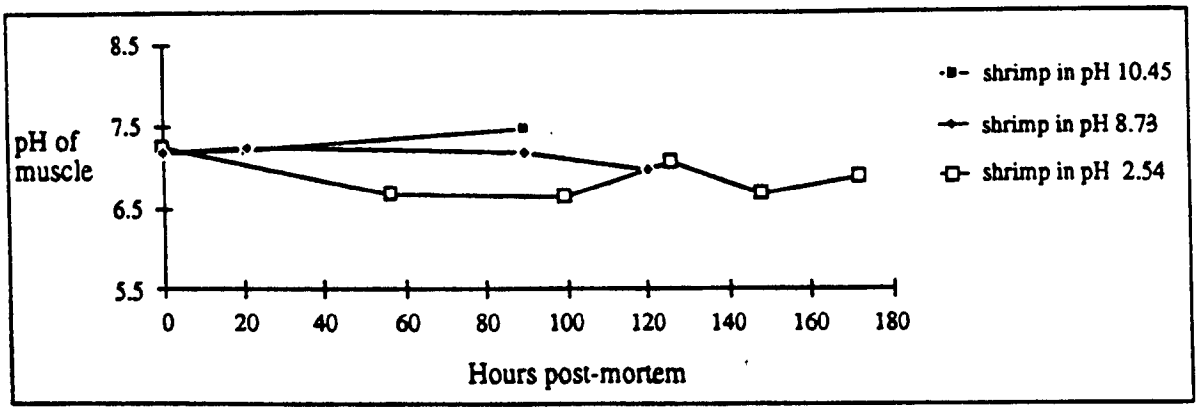
Text figure 8.1: Two possible mechanism of producing 'zonal phosphatization' of soft tissues in fish carcasses.
a) Concentration of dissolved phosphorus decreases with depth in the carcass. b) Apatite is not stable either at the pH of the external environment or of the decaying fish; it is however stable where they mix. c) Apatite is not stable either at the pH of the external environment or in the zone where this mixes with the decaying fish itself. d) The result of the gradual decrease in the concentration of phosphorus with depth into the carcass and the provision of a favourable environment for the precipitation of HAP only in a specific zone of the carcass, is that the most heavily phosphatized tissues are some depth beneath the dermis.

diminishing above and below this zone (fig. 8.1). Phosphorus was not identified in the surrounding concretion. Since apatite precipitation is extremely pH- and supersaturation-sensitive (Nancollas, 1982), it is logical to conclude that the most heavily mineralized zone represents the region which provided the most favourable environment for its precipitation. Since this is not simply those tissues which were nearest to the source of dissolved phosphorus (assuming an external source, see Section 7.2.1), then either:

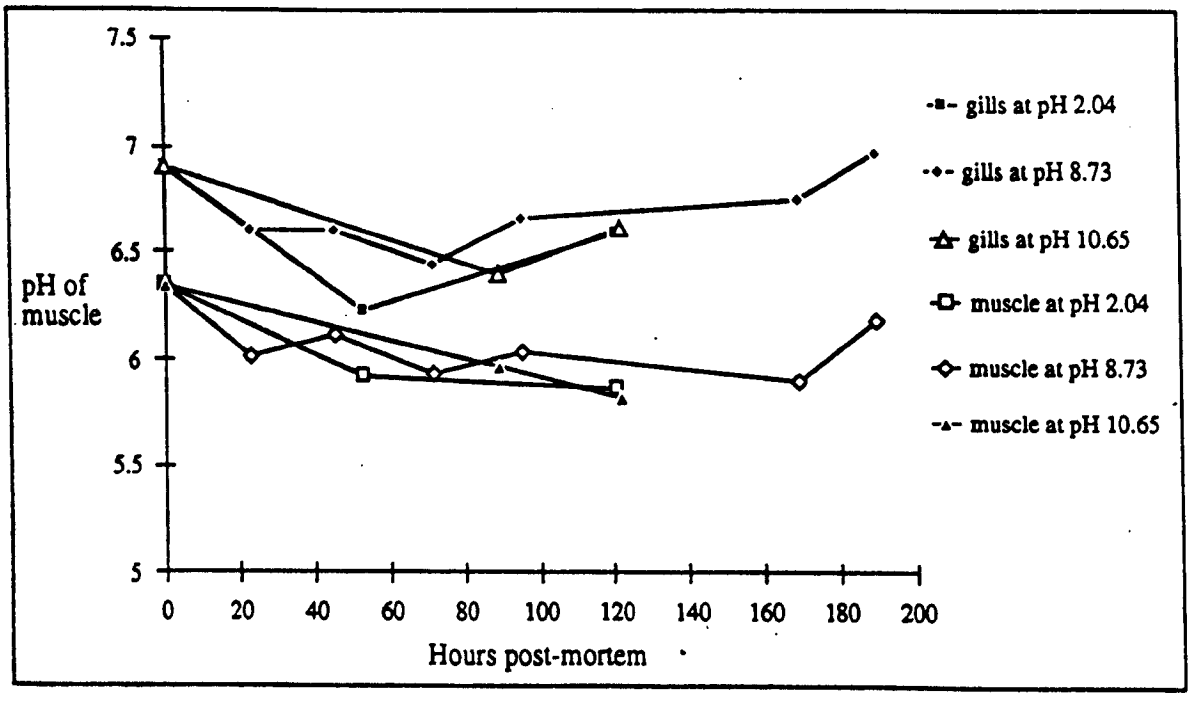
a) the microenvironment produced by the decaying fish *alone* was not ideal for the precipitation of apatite; optimum conditions for phosphatization were only created in a specific zone of tissue where the external environment mixed with that of the decaying fish (text fig. 8.1a, b, d); or that

b) the microenvironment produced by the decaying fish was ideal for the precipitation of apatite but the influence of the external environment in the most peripheral tissues of the carcass prevented its precipitation here. Only beyond the area influenced (i.e. slightly deeper in the organism) was apatite stable. Therefore mineralization was restricted to a zone adjacent to the area influenced by the external environment where the highest levels of dissolved phosphorus occurred (text fig. 8.1a, c, d).

Since apatite is not a conspicuous authigenic phase in the sediments surrounding the fossil fish of the Romualdo Member, it would appear that at least up until the point of phosphatization, the decomposing fish were capable of maintaining a chemistry (and in particular a pH) favourable to the precipitation of apatite and distinct from that of the external environment (except in the outer few microns of their body). This is supported by the results of actualistic decay experiments in which the evolution in the pH of decaying shrimps (*Crangon* sp.) and fish (*Gadus* sp.) when exposed to pHs as high as 10.65 and as low as 2.04 was very little different to that of carcasses decomposing under 'normal' (i.e. pH ≈8.7) marine conditions (text figs. 8.2 and 8.3). Indeed, even tissues as permeable and as 'exposed' as the gills of the fish were capable of remaining relatively unaffected (see text fig. 8.3). The pH of these tissues was sampled in the manner already described in Section 6.2.1.2.



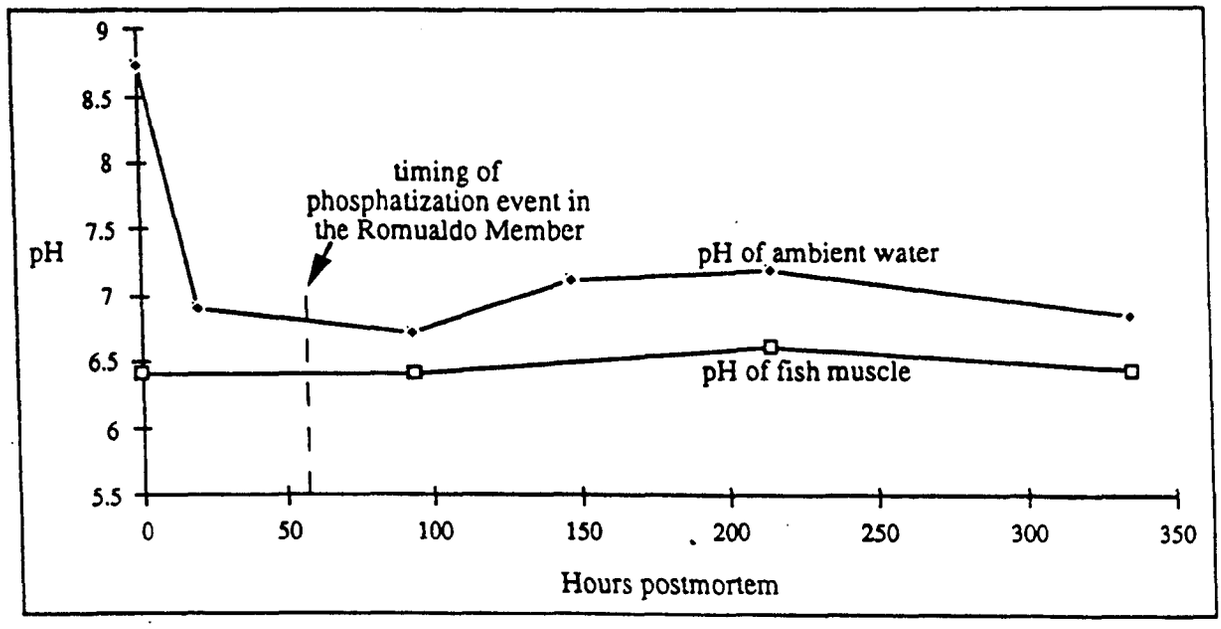
Text figure 8.2: The stability in pH of shrimp's muscle (*Crangon* sp.) postmortem under extremes in ambient pH (pHs 10.45 and 2.54) at 21^{0}C, 3.5% salinity (water volume ≈35 times that of the shrimps).



Text figure 8.3: The stability in pH of the gills and skeletal muscle of fish (*Gadus* sp.) under extreme pHs (pHs 2.04 and 10.65) at 21^{0}C, 3.5% salinity (water volume 8 times that of the carcass).

Martill (1988, p11), and Briggs and Kear (1993a) have proposed that microbial mats enshrouding decaying carcasses may have played an important role in the phosphatization of soft tissues by constraining the organisms' decay-induced microenvironments. Actualistic experiments in which fish (*Gadus* sp.) were placed in extremely restricted volumes of water (modelling confinement by a microbial mat) suggest that under such conditions, the interstitial water of the sediment would have rapidly approached or even acquired the pH of the carcass itself (text fig. 8.4). Apatite would therefore be expected to precipitate *en mass* external to the carcass. This has not been observed in any of the fossil material examined

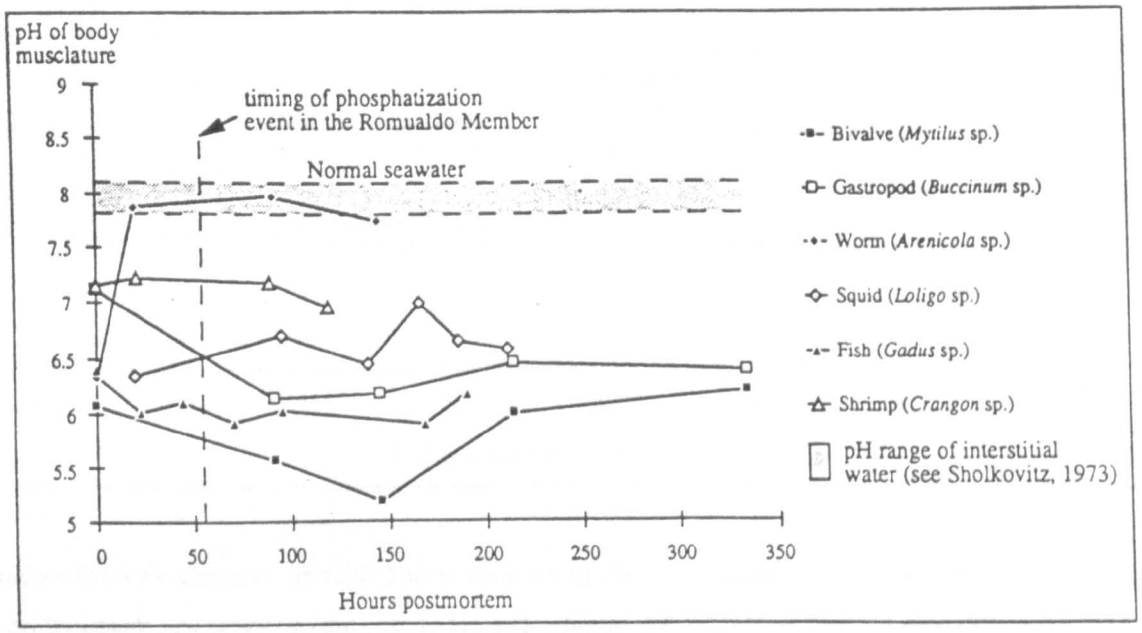
(see Chapters 4 and 5) and thus microbial mats may not be as important as has previously been supposed.



Text figure 8.4: Postmortem evolution in the pH of the muscle of *Gadus* sp. (volume ≈1 litre) within a restricted volume of water (600ml) at 21^{0}C, 3.5% salinity. The pH of the ambient water was measured (as in the following experimens) by drawing off a 10ml sample into a small vessel. The reading was taken after 3-4 minutes to permit the value to stabilise.

Clearly, decaying fish offer an environment suitable for the precipitation of apatite. But do all organisms? Measurements of the evolution in pH of the musculature of different groups of decaying organisms indicates that they create specific and predictable microenvironments (text fig. 8.5) which are largely independent of the pH of the ambient water (see text figs. 8.2 and 8.3). These microenvironments may be maintained for considerable periods of time in some organisms (e.g. bivalves and squid). Contrary to the pH of the water surrounding carcasses (Briggs and Kear, 1993b, fig. 6), the pH of the carcasses themselves does not appear to be greatly affected by the extent to which metabolites and in particular oxygen may diffuse into and out of the system. The pH of fish decomposed within a number of different volumes of water (and therefore probably quite different rates of diffusion between the reaction vessel and the atmosphere) were relatively constant (see text fig. 7.3).

The enormous difference in pH between the decaying tissues of different groups of organisms (see text fig. 8.5) suggests that the microenvironments created by the decay of some groups may be beyond the stability field for apatite. There has been some debate as to what are the optimum conditions for the precipitation of apatite. Allison (1988a, p338; 1988d, p407) concluded apatite to be stable under alkaline reducing conditions based on



Text figure 8.5: Postmortem evolution in the pH of muscle from selected organisms decayed at 21^0C, 3.5% salinity, and in a volume of water ≈5 times that of their own.

Guldbrandsen's (1969) stability fields for apatite, and on the occurrence of high pH microenvironments around decomposing organisms (Berner, 1968a). Similarly, Stumm and Morgan (1970) and Burnett (1977) suggest the stability field of apatite to be largest at high pHs. However, Krumbein and Garrels (1952), Kramer (1964), Berge (1972), Nathan and Lucas (1976), Nathan and Sass (1981), and Coleman (1985) have suggested that lower pHs are more likely to promote the precipitation of apatite. This is supported by text figure 8.5 which indicates that at the time of phosphatization (≈55hrs postmortem, see Section 6.3.2), most decomposing organisms whose fossil representatives yield phosphatized soft tissues, offer an environment of significantly lower pH than either open marine water or the interstitial fluids of anoxic sediments (see Baturin, 1972; Sholkovitz, 1973). Only later do decomposing organisms have the high pHs as recorded by Berner (1968a, fig. 1). This is consistent with the model presented by Martill (1988) for the Romualdo Lagoon.

Assuming therefore low pHs to enhance the probability of soft tissues being phosphatized, the differences in the pH profiles of different groups of decaying organisms (text figure 8.5) provides an explanation for the preferential phosphatization of soft tissues in certain organisms. For example, it is clear that since worms (e.g. *Arenicola* sp.) are incapable of maintaining a microenvironment independent to that of the ambient fluid, they are unlikely to offer a favourable site for the precipitation of apatite. In contrast, all (except for the gastropod - *Buccinum* sp.) of the other organisms under examination are capable of maintaining a distinct microenvironment, thus providing an explanation for the frequent preservation of phosphatized soft tissues in fossil representatives of these groups. The absence of any recorded examples of gastropods with phosphatized soft tissues requires an alternative explanation (see below).

Repeat experiments with different species each of fish (*Gadus* sp. and *Clupea* sp.) and gastropods (*Buccinum* sp. and *Nucella* sp.) suggests that to a first approximation, the microenvironments of closely related organisms are very similar. This probably reflects similarities in gross composition and structure, and thus the rate at which closely related organisms decay (although see Allison, 1990b), are invaded by microbes, and permit diffusion of water and electrolytes across their body walls. In a simple system, this would suggest that if one individual of a group of organisms contains phosphatized soft tissues, all representatives of that group should. The differential phosphatization of fish from the Romualdo Member demonstrates that this is certainly not the case. Other variables are implicated.

It is tempting to suggest that one controlling factor in the differential mineralization of related organisms is the presence or absence of specific chemicals which stimulate or inhibit phosphatization. For example, the ammonia-rich buoyancy mechanism utilized by squid (Fretter, 1968) may explain their disproportional phosphatization relative to ammonites (which use their phragmocone for the same purpose). Similarly, the frequency with which soft tissues are preserved in sharks and rays compared to their abundance as fossils (see table 8.1) suggests a causative relationship (perhaps related to the presence of squalene in chondrichthyans, Moyle and Cech, 1982). The presence of an inhibitory chemical in the soft

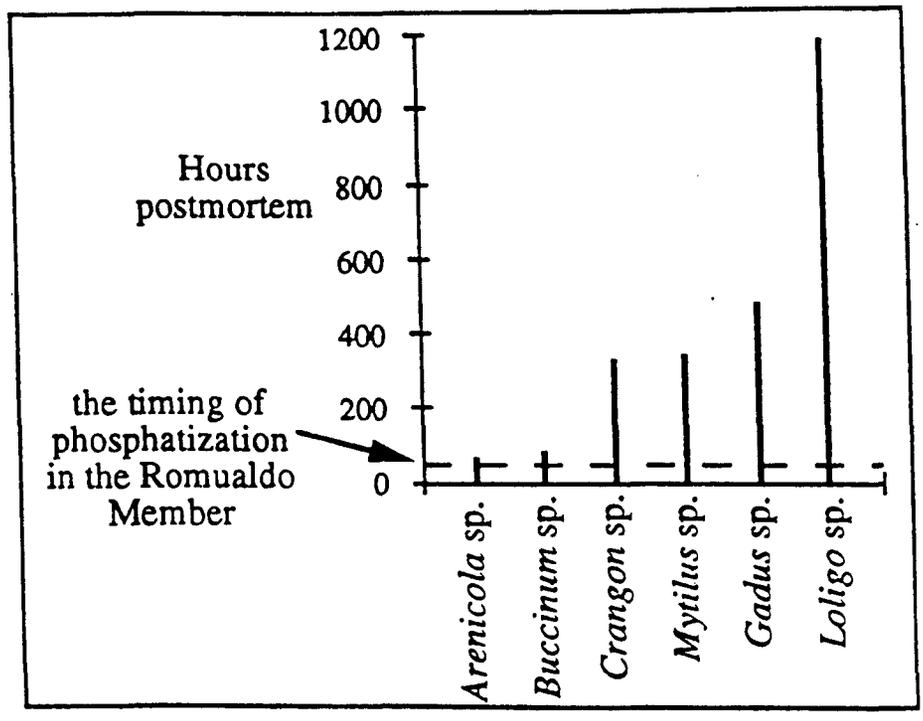
tissues of *Vinctifer* from the Romualdo Member *may* explain the absence of phosphatized soft tissues in this fish. Certainly, the concentration of known inhibitors to the precipitation of apatite (see Section 3.3.5) such as Mg^{2+}, do vary considerably between related organisms (see Vinogradov, 1953).

3) Decay rate: Actualistic taphonomic experiments demonstrate different organisms to decay at different rates. Text figure 8.6 gives an indication of the rates of decay of six different groups of organisms (under identical conditions) relative to the timing of mineralization in the Romualdo Member (see Chapter 6). Each bar is a qualitative measurement of the period of time over which portions of the body musculature of that organism were cohesive enough to be retrieved intact from its decay vessel. Beyond this point the tissues were so extensively degraded that they "liquified" with the slightest disturbance. There is a clear correlation between the rate at which different groups of organisms decay (see text fig. 8.6) and the frequency with which phosphatized soft tissues occur in those groups (see table 8.1). For example, the soft tissues of worms (e.g. *Arenicola* sp.) and gastropods (e.g. *Buccinum* sp.) decay rapidly and are relatively scarce as fossils, whilst those of fish (*Gadus* sp.) and squid (*Loligo* sp.) are relatively resilient, and are therefore considerably more abundant as fossils.

The rate of decay of Recent worms (e.g. *Arenicola* sp.) (see text fig. 8.6) relative to my estimate for the timing of mineralization in the Romualdo Member (see Section 6.3.2) suggests that even if worms had been an important component of the Romualdo Member's fauna, their soft tissues would not have been present at the time of phosphatization.

Since body musculature has a *similar* composition in all organisms (see Vinogradov, 1953), variations in the rate of its decay in different organisms (see text fig. 8.6) are probably related to differences in the ease with which microbes may gain access to it. For example, the body musculature of worms is protected only by a very thin cuticle and is therefore prone to microbial infestation. Similarly, the susceptibility of muscle in gastropods and bivalves is reflected in the speed at which these organisms decay. In contrast, the skeletal muscle of fish may only be reached once the scales and relatively thick dermis are

breached. The rate at which the skeletal muscle of fish decompose is therefore correspondingly slow.



Text figure 8.6: The rate of decay of various organisms (21^{0}C, 3.5% salinity, in ≈8 times their own volume of water) relative to the timing of phosphatization in the Romualdo Member. Each bar represents the period over which the body musculature of that particular organism retained sufficient integrity to be retrieved from the experimental vessel.

The body musculature of squid is protected from microbial invasion only by a very thin dermis. Nevertheless, squid decay extremely slowly (see text fig. 8.6). This anomaly is more likely related to the release of antibiotic chemicals associated with their ammonia-regulated buoyancy mechanism.

A major determining factor in an organism's potential to become phosphatized would therefore appear to be the rate at which it decays relative to the timing of the mineralization event. Decay rate is directly controlled by temperature (Schäfer, 1972; Elder, 1985; Elder and Smith, 1988; Kidwell and Baumiller, 1990). It is therefore possible that the presence of phosphatized soft tissues in a certain organism from one deposit but not in a closely related organism in another deposit may be the result of differences in the two deposits palaeotemperatures. This effect would be most obvious in rapidly decaying organisms (e.g. worms), thus providing a possible explanation for the preferential phosphatization of soft tissues in Annelida from certain deposits (e.g. the Solnhofen Limestone, Lombardische

Kieselkalk Formation, and the Granton shrimp bed) and their absence in other deposits (e.g. the Romualdo Member, Portland Roach, and the Glencartholm Volcanic Beds). It is conceivable that the latter deposits were laid down under slightly warmer conditions than the former.

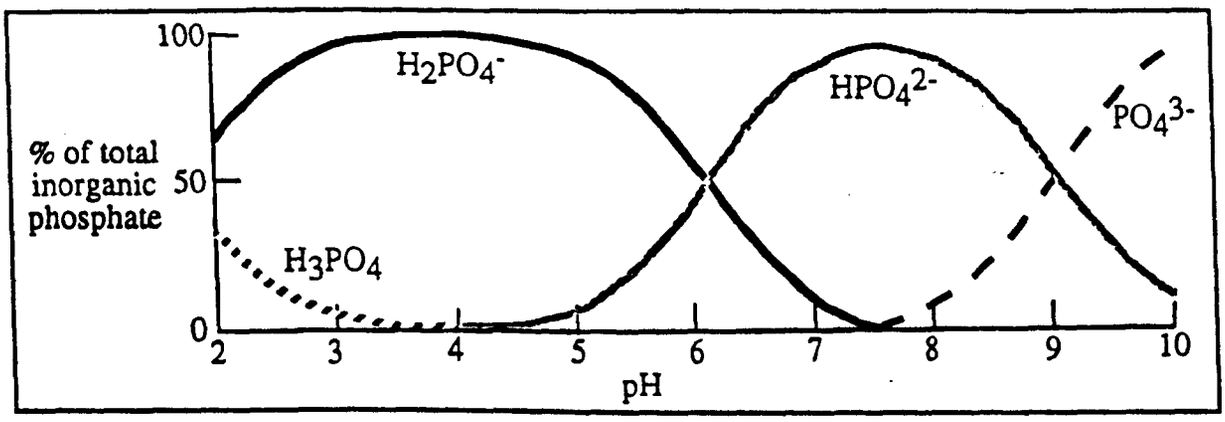
4) Position in the sediment: The position of fish in the sedimentary pile of the Romualdo Lagoon relative to the redox boundary determined the concentration of adsorbed phosphorus to which they were exposed; only those fish which did not sink excessively into the soupy substrate had access to sufficient phosphorus to promote phosphatization (see Section 7.2). The position of carcasses in the sediments of other deposits may have been an important control on the likelihood of their soft tissues being phosphatized. Indeed, assuming most fish of the same Class to have had a *similar* chemical composition (see Vinogradov, 1953, tables 290, 292, 295-297), to have decayed at more or less the same rate, and to have created a similar microenvironment during decay (see above), their position of rest in the sediment provides one of the only explanations for the occurrence of phosphatized soft tissues in one species and not in another from the same deposit.

For example, without proposing that *Vinctifer* (a 'heavily armoured' fish, see Maisey, 1991, pp170-189) sank to greater depths into the soupy substrate of the Romualdo Lagoon than flat-lying specimens of *Notelops* and *Rhacolepis*, it is extremely difficult to account for the preferential phosphatization of soft tissues in the latter two genera (see table 4.1). The relative density of carcasses may also account for the general absence of phosphatized soft tissues in extremely large fish (>1m) from the Romualdo Member. Even representatives of species which frequently contain fossilized soft tissues when immature rarely do when mature (e.g. *Notelops*); their greater mass:surface area ratio would have prevented them from remaining in the phosphorus-rich zone.

The position of carcasses in the sedimentary profile also provides an explanation for the absence of phosphatized soft tissues in pycnodonts from the Romualdo Member but their presence in pycnodonts from the Solnhofen Limestone (Reis, 1898). Whereas the upper few tens of centimetres of sediment in the Romualdo Member were soupy (see Section 2.5.4), the sediment surface of the Solnhofen Limestone was covered with stiff bacterial mats

(Barthel *et al.*, 1990). These mats were certainly firm enough to permit ammonites to role across their surfaces (Seilacher *et al.*, 1985, Plate 1). The Solnhofen Limestone was therefore far more capable of maintaining the relatively heavily 'armoured' pycnodonts in the upper few centimetres of the sediment (where I propose phosphorus was secondarily enriched by adsorption onto ferric hydroxides) than the soupy sediment of the Romualdo Lagoon.

5) Rates of phosphate diffusion: Phosphorus probably invades the soft tissues of different groups of organisms at different rates. Indeed, certain groups of organisms may be completely impermeable to dissolved phosphorus. This therefore provides a potential mechanism of differential mineralization.



Text figure 8.7: Dissociation of phosphoric acid over a range of pHs in normal seawater (after Atlas, 1975). See text for details.

Mann *et al.* (1983) have demonstrated experimentally that the rate at which dissolved phosphorus diffuses across biological membranes is related to pH-associated shifts in the disassociation of phosphoric acid (text fig. 8.7). Due to the greater charge of PO_4^{3-} compared to $H_2PO_4^-$, rates of phosphorus diffusion across the highly hydrophobic (or water repulsive) regions of lipid membranes at pHs characteristic of PO_4^{3-} (see text fig. 8.7) will be trivial relative to those pHs characteristic of $H_2PO_4^-$. At the pH of decaying fish, the approximate composition of phosphorus-rich solutions will be 25% HPO_4^{2-} and 75%

$H_2PO_4^-$, whereas at the pH characteristic of decaying worms, 5% will be PO_4^{3-} and 95% HPO_4^{2-} (see text fig. 8.7). Therefore, the soft tissues of fish are likely to be invaded by an external source of dissolved phosphorus much more rapidly than those of worms, and thus have a greater chance of being phosphatized. This is consistent with the relative abundance of phosphatized soft tissues in fossil worms and fish (see table 8.1).

6) Carcass size: Another important variable which has received little attention is the effect that the size of the carcass has on its potential for phosphatization. Of particular interest is: a) the size of the decay halo of large organisms relative to their mass, and b) the rate of decay of different sized organisms. These are examined separately:

a) *Relative size of decay halo:* Assuming an adsorbed source of phosphorus, at a said time postmortem, the volume of sediment reduced by the decay of a large organism will, in proportion to its mass, be smaller than that of a smaller carcass. Therefore, although the quantity of adsorbed phosphorus available to the large carcass will be greater than that of the smaller organism, it will be smaller relative to the mass of that organism. This may partly explain why the soft tissues of large fish (>1m) in the Romualdo Member are only rarely phosphatized. The *relatively* small decay-halos of large carcasses may however, be counteracted by the enormous internal reserve of phosphorus in these organisms (which must be released by microbes). This may account for the relatively frequent occurrence of phosphatized soft tissues in ichthyosaurs (see table 8.1).

As mentioned in Section 7.3, there would also appear to be a lower limitation on the size of organisms which may be phosphatized by phosphorus adsorbed onto clay particles. Small organisms such as crustaceans *may* not be capable of reducing a large enough volume of sediment to sufficiently saturate the interstitial waters with dissolved phosphorus.

2) *Decay rate:* Allison *et al.* (1991) have suggested that large carcasses with their greater surface area to mass ratio, will decay slower than smaller ones due to the less effective diffusion of electron donors from the external environment to the respiring microbes. This is supported by the dramatic increase in the rate of decay of chitin reported by Chan (1970) when the size of the chitin particles was reduced. This suggests that the soft tissues of large

carcasses should have an inherently greater chance of being phosphatized since they are available to be mineralized over a greater period of time.

7) Environmental controls: Phosphogenesis is restricted to specific environments, one characteristic of which is inhospitable bottom waters (see Section 7.4). Therefore, pelagic organisms (e.g. fish and squid) and those which are capable of rapid colonization and/or are tolerant to adverse conditions (e.g. some crustaceans), are likely to have the greatest chance of being phosphatized. It is exactly these organisms whose soft tissues are most frequently preserved (see table 8.1). Although other organisms may be suited to phosphatization, they may for ecological reason very rarely be exposed to the correct environmental/sedimentological conditions, and thus rarely contain phosphatized soft tissues.

As discussed in Section 4.2.2.1, the abundance of individuals of a certain group of organisms is also an important control on the probability phosphatized soft tissues occurring in that group. For example, since only a few terrestrial organisms have been recorded from deposits yielding phosphatized soft tissues, the chances of their soft tissues being fossilized are extremely remote.

Interestingly, the soft tissues of closely related organisms may be phosphatized under superficially (see table 7.1) very different environmental conditions (e.g. the fish of the Romualdo Member and the Solnhofen Limestone). Since different environmental settings imply differences in the concentration of phosphorus available, sediment consistency, and porosity, it would appear that closely related organisms may be phosphatized under a variety of conditions (*within certain limitations*). That is, phosphatization is not the result of a single combination of variables, but rather the consequence of a compromise between a number of different factors. For example, a low concentration of dissolved phosphorus in the sediment of one deposit may be counteracted in another deposit by a relative increase in porosity (increasing the volume of sediment available to be reduced), a reduction in the rate of decay of the organism (by varying the temperature and salinity of the interstitial fluids, see text figs. 6.5 and 6.6 respectively), and/or by the decomposing carcass containing a greater internal concentration of phosphorus.

In apparent contradiction, it would appear that even when closely related organisms occur in sediments which were deposited under comparable environmental conditions, there is no guarantee that soft tissues will be phosphatized in both. This is exemplified by the Cordillera de Domeyko (see Section 5.2.6), the Oxford Clay Formation (see Section 5.2.7), and the Lower Lias (see Section 5.2.9) which despite having similar palaeoenvironments and faunas, do not yield phosphatized soft tissues in the same organisms. This illustrates just how stringent the environmental parameters are for phosphatization. Indeed, there are countless other deposits world-wide with a similar fauna and sedimentology which don't contain phosphatized soft tissues at all. The palaeoenvironmental specificity of soft tissue phosphatization has recently been emphasized by Briggs and Kear (1993a).

CONCLUSIONS: Phosphatization of soft tissues is a complex (and not easily predicted) interplay between a number of variables, the combination of which differs for each organism. A source of dissolved phosphorus is not in itself adequate to induce the phosphatization of decaying organisms.

Certain organisms (e.g. crustaceans, bivalves with phosphatic gill supports, squid, and fish) appear to be somewhat 'preconditioned' for phosphatization. They contain a relatively high concentration of phosphorus in their soft tissues, decay fairly slowly, and create a decay halo with a pH distinct from that of normal sea water and most interstitial fluids. Preferential phosphatization of soft tissues within individuals of these groups of organisms appears to be most closely related to the concentration of phosphorus in their tissues, the relative abundance of individuals, and their position within the sediment with respect to the redox boundary.

It is interesting to note that despite the frequent abundance of plant material in the deposits listed in table 8.1, plants are only very rarely phosphatized. To my knowledge, only a few deposits (e.g. the Tertiary of the Pacific Ocean [Goldberg and Parker, 1960], the Eocene London Clay [Balson, 1980]) yield phosphatized plant remains. Since plants produce an acidic microenvironment on decomposition (Bushiniski, 1966), the rarity with which they

are phosphatized is unlikely to be pH related. Instead, their rarity may be a consequence of one or more of three other factors. These are:

a) The extremely slow rate at which most plants decay (see Allison and Briggs, 1991a, pp31-32). This may limit the concentration of phosphorus which can build up around the plants at any one time, by extending the period over which adsorbed phosphorus is released from the sediment by the plants' decay-induced microenvironment.

b) Plants may not offer many (or any) suitable sites for the nucleation of HAP.

c) Phosphorus is relatively impoverished in plants. This prevents plants from subsidizing the external source of phosphorus to the extent that decaying animals may do (see Chapter 7).

8.2.2 DIFFERENTIAL PHOSPHATIZATION OF ORGANS AND TISSUES WITHIN FISH OF THE ROMUALDO MEMBER

Only the dermis, body musculature, gills, and alimentary tract are preserved in abundance in fish from the Romualdo Member; most other organs are extremely rare. There are a number of possible explanations for this, each of which is discussed separately below:

1) Tissue position: EDAX elemental maps (see Appendix 3i) across fish from the Romualdo Member indicate a clear gradient in the abundance of phosphorus from a high at the periphery just beneath the dermis, to a low at some greater depth within the fish (see Section 7.2). This suggests dissolved phosphorus and ?Ca^{2+} to have invaded the carcasses from an external source by simple ionic diffusion encouraged by their continual removal from solution at the sites of mineralization. Elemental maps displaying marked differences in the density of phosphatization of muscle fibres on either side of myosepta (fig. 8.2) indicate certain membranes to have represented relatively impermeable barriers. This is supported by the limited width of tissue which is phosphatized in most of the fish (never >1cm).

Microbial infestation is the most common mechanism by which soft tissues are phosphatized (see text fig. 5.6). Therefore, externally located organs may also have had a greater chance of being fossilized than those located deeper in the carcass because of their

greater susceptibility to microbial infestation. The relationship between the density of microbial infestation and that of authigenic mineralization was also recognized by Allison (1988c, p152).

The position of tissues relative to the external source of phosphorus and to populations of microbes were two of the most important factors which determined which tissues did, and which did not become phosphatized in fish from the Romualdo Member. Rapid proliferation of microbes and close proximity to the source of lattice ions permitted even the most delicate of organs in fish from the Romualdo Member to be preserved (e.g. the gills, see Section 4.2.2.1).

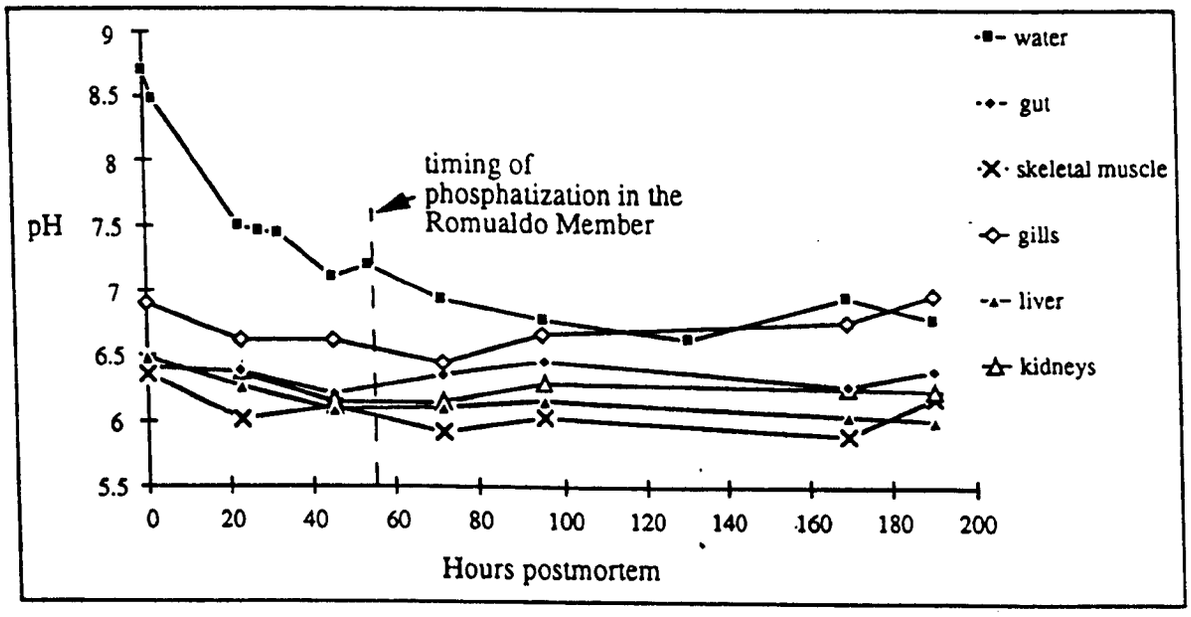
2) Concentration of phosphorus in the organs: Martill (1989b, p5) and Schultze (1989) have both suggested that metabolically active tissues (i.e. those rich in blood and dissolved phosphorus) have the greatest potential for phosphatization. Thus, the gills, musculature and stomach are most frequently fossilized. However, as discussed above, the preservation of these tissues may simply reflect their accessibility to the external environment.

According to Vinogradov (1953, p512), the sex organs and livers of fishes contain the greatest concentration of phosphorus (0.45wt%+P wet tissue compared with ~0.25wt%P in muscle). The extreme rarity of these organs in fish from the Romualdo Member suggests to some extent (other factors may be involved) that the concentration of phosphorus in organs is no indication of the frequency with which they become *inorganically* phosphatized.

3) Decay rate: Allison (1988d, pp406-407) has suggested that due to the slower rate of their decay, the mantles of teuthids from Christian Malford (Oxford Clay Formation, see Section 5.2.7) are preferentially preserved relative to the arms of these organisms. This results from the greater surface area to mass ratio of the arms, and therefore the greater rate at which essential metabolites may diffuse to the invading microbes (see Section 8.2.1). Although I envisage proximity to the source of dissolved phosphorus to have been the most important control on the fossilization of soft tissues in fish from the Romualdo Member (see above), the absence of many externally located organs (e.g. the eyes) even in heavily

phosphatized fish, suggests that differences in the rate of decay of organs may also have been of some importance.

Taphonomic experiments indicate the various organs of fish to decay in a predictable sequence. This in order of increasing stability is: nervous tissues (e.g. brain) > gills and eyes > liver and stomach > kidneys > skin > muscle. The most volatile tissues (e.g. the brain) may be completely decomposed several days in advance of the most taphonomically stable one - skeletal muscle (text fig. 8.8). These differences probably reflect variations in the average age, composition and size of the constituent cells of each organ; the relative abundance and timing of release of their lysosomal enzymes; and their proximity to the external environment and therefore susceptibility to osmotic fluctuations and bacterial infestations.



Text figure 8.8: Postmortem evolution in the pH of various organs of fish (*Gadus* sp.) at 21^{0}C, 3.5% salinity, and in 5 times the carcasses' own volume of water.

According to my estimate for the timing of phosphatization of soft tissues in the Romualdo Lagoon (see Chapter 6), the brain, eyes, and blood cells of fish would have been in a state of considerable decay at the point of mineralization. This may account for the rarity of fossil examples of these tissues. It does not however, explain the absence of many other tissues in the fish of the Romualdo Member. Most of the major organs of fish remain in a fairly good state of preservation in taphonomic experiments far beyond the timing of

mineralization in the Romualdo Member (≈55hrs). An alternative explanation for their absence in the fossil fish is therefore required (see above and below).

The alimentary tracts of fish from the Romualdo Member are commonly preserved (see table 4.1). This implies that they had access to a fairly reliable source of dissolved phosphorus (the gut contents and/or the external environment). It is therefore, rather surprising that the closely associated sex organs, kidneys, and liver (which on the basis of taphonomic experiments are assumed to have been present at the time of mineralization) are not phosphatized too. This is especially surprising in fish from the Romualdo Member whose body walls have ruptured and whose internal organs would therefore have been exposed to the external environment. Since isolation from the external environment and the premature decay of these organs cannot be assumed, I must conclude that many (and in particular the kidneys) were inherently resistant to the nucleation of apatite. In humans at least, kidneys are prone to pathological biomineralization during life, and are therefore endowed with certain inhibitors (Lehninger, 1983). It is possible that these inhibitors remained active in the fish of the Romualdo Lagoon even after death (see Section 8.2.1).

4) Tissue pH: In Section 8.2.1, I argued that differences in the pH of decomposing organisms may have resulted in some becoming preferentially phosphatized. Although individual organs are capable of maintaining an independent pH during decay, this is very little different to that of adjacent organs within the same fish (text fig. 8.8). Indeed, the pH of skeletal muscle (one of the most frequently preserved tissues in the fish of the Romualdo Member) and the kidneys and liver (both of which have never been observed in the Romualdo Member) of decaying fish are very similar. Therefore, differences in the pH of tissues were unlikely to have induced the extent of differential phosphatization that is observed in the fish of the Romualdo Member. However, since the morphology of inorganic crystal microfabrics are very sensitive to pH (see Section 3.3.5), these differences, when in combination with the variations in the concentration of dissolved phosphorus across the carcasses (see above), may have been responsible for the enormous variety of crystal microfabrics which occur within individual fish (see Chapter 3).

8.2.3 DIFFERENTIAL PHOSPHATIZATION OF CELLS, ORGANELLES AND BIOMOLECULES WITHIN THE FISH OF THE ROMUALDO MEMBER

An understanding of the role that the cells and their constituent biomolecules have in facilitating (or even inducing) phosphatization is fundamental to the development of a realistic model of inorganic postmortem soft tissue phosphatization. From the evidence presented in Chapter 4, it is clear that organic substrates in the decaying fish of the Romualdo Member played two roles. They either 1) participated indirectly in phosphatization by comprising a membrane or barrier capable of maintaining the chemistry of the enclosed space independent to that of the immediate vicinity; or 2) participated directly in fossilization by providing a substrate suitable for the heterogeneous nucleation of apatite. Specific examples of both are discussed below:

1) INDIRECT PARTICIPATION: Evidence for the maintainance of chemically isolated compartments during phosphatization is abundant in the soft tissues of fish from the Romualdo Member. Particularly common are the internal moulds of organelles within cells whose cytosol and endoplasmic reticulum remain completely unmineralized, but whose plasma membrane is preserved as an external coating (fig. 8.3). In such cases, assuming an external source of phosphorus (see Section 7.2.1), the existence of at least three chemically isolated compartments must be conceded: two providing a favourable environment for the precipitation of apatite (the organelles and extracellular space), and the other (the intracellular compartment) being incapable of inducing phosphatization. A similar situation is invoked for the phosphatized nuclei and extracellular space of epidermal cells (see fig. 4.3).

Less direct evidence for compartments having maintained an independent chemistry postmortem is given by the absence of certain subcellular bodies within otherwise 'densely' phosphatized tissues. For example, in striated muscle the 'ghosted' preservation of mitochondria (see fig. 4.21), nuclei (see fig.4.18), T-tubules (see fig. 4.24), and capillary loops (see fig. 4.23) implies that these structures were present at the time of mineralization but were not phosphatized due to their chemical isolation. In a muscle fibre figured by Schultze (1989, Plate 4, fig. 1) from the Cordillera de Domeyko, a nucleus is preserved as

an internal cast by microgranular apatite whilst the fibre itself is replaced rather imprecisely by microspheres. This suggests that the muscle cell maintained at least two distinct chemical compartments each of which was suited to the precipitation of a different phosphate phase. Similarly, the absence of pilaster cells in the gill apparatus of fish from the Romualdo Member (see Section 4.2.2.1), and non-mineralization of the squamous epithelium of the digestive tract (see fig. 4.33) despite being adjacent to other heavily mineralized cells, suggests they retained an independent chemical identity throughout mineralization.

In some fossil tissues, it appears that the control exerted over mineralization by membranes diminished with time. For example, in striated muscle fibres in which each half of every sarcomere is replaced by a single microsphere (see fig. 4.13), it would appear that the membranes were initially important in constraining the precipitation of ACP to specific sites (the sarcomeres). However, since the sarcomeres' original cuboidal morphologies are no longer preserved in the fossil, the integrity of the membranes must have been somewhat compromised during the conversion of the ACP to HAP. This suggests that delays in the acquisition of the fossil's final mineralogy (either due to phase transitions or the presence of nucleation inhibitors) seriously affects the resolution of detail preserved.

2) DIRECT PARTICIPATION: Evidence for the direct participation of organic matrices in phosphatization is widespread. Indeed, the absence of an amorphous precursor phase (e.g. ACP) and the discriminate nature with which mineralization took place in itself implies apatite to have nucleated directly onto certain organic substrates. The most convincing evidence however, is given by those tissues which are replaced by equi-sized crystallites with a preferred orientation. Examples include fish epidermis (see figs. 4.3 and 4.4), and the exoskeleton of decapod crustaceans. In these tissues at least, the processes of crystal nucleation and growth were strictly mediated by the matrix.

It is clear from a detailed examination of the fossil material (see Chapter 4) that not all organic substrates were capable of inducing HAP nucleation; certain matrices are preferentially mineralized over others. This is evident even within individual cells. Commonly, both the plasma membrane and the organelles are preserved as internal moulds, their external surfaces remaining entirely unmineralized (see figs. 4.50, 4.51). Since the

internal surface of the plasma membrane and the external surface of the organelles enclose the same intracellular space, they, the cytosol, and the endoplasmic reticulum are likely to have been exposed to the same chemical environment. Under conditions of simple HAP precipitation where every substrate can nucleate apatite, all of these components would be phosphatized. This is clearly not the case.

Striated muscle fibres provide further evidence for the specificity of the mineralizing process to certain biomolecular substrates. In most cases, phosphatization is confined predominantly to the area occupied by the actin filaments; the Z-discs, A-bands, and sarcolemma usually remain entirely unmineralized (see fig. 6.17). Whilst the absence of phosphatized A-bands is interpreted to be a taphonomic phenomenon (see Section 6.2.1.2), it is difficult to interpret the non-mineralization of the associated Z-discs and sarcolemma (which would have been exposed to the same chemical environment) as anything other than their inability to provide a suitable site for the nucleation of apatite.

8.3 INORGANIC POSTMORTEM PHOSPHATIZATION

Lowenstam (1981) divided biomineralization into two fundamentally different groups based on the degree of biological control exerted over the process. These are:

1) "Biologically-induced mineralization" which describes systems in which mineral precipitation results from the interaction of living organisms with their surrounding physical environments. The biological systems exercise little control over the type and habit of mineral deposited, and may not even be involved in the concentration of the mineral constituents. The system simply provides a site for the precipitation of the mineral. Classic examples are well documented from a number of unicellular organisms, and usually involve shifts in equilibrium between dissolved ions surrounding (or inside) the biological systems caused by the release of metabolic bi-products from the organisms (e.g. Ennever *et al.*, 1981).

2) "Organic matrix-mediated mineralization" encompasses those systems in which the nucleation, growth and microarchitecture of a specific mineral is precisely regulated by a genetically programmed organic matrix. It *may* involve the precipitation of a crystal in one locality and its transportation to another where further growth or modification can take place.

This is exemplified by the mineralization of bone collagen (see Francillon-Vieillot et al., 1990).

Schultze (1989) and Martill (1990a) have both invoked specific models of biomineralization in an attempt to account for the exceptional preservation of soft tissues in fossil deposits. Clearly, gross physical and mineralogical similarities do exist between the inorganic products of postmortem mineralization and those of biomineralization. For example, both systems must explain the formation of an inorganic phase in or around biological molecules in terms of physical parameters such as pH, level of supersaturation, inhibitors, and the availability of suitable substrates for nucleation. Indeed, there is no reason to doubt that the ultimate control on mineralization (the mechanisms of crystal nucleation, see Mann, 1983) are any different in the two processes. However, a number of factors negate traditional models of biomineralization (without modification) as being capable of providing an accurate description of the process of inorganic postmortem phosphatization:

Principally, structures indicative of the initial stages of decay are obvious in the soft tissues of the majority of lagerstätten examined in this study (see Section 6.2), suggesting mineral deposition proceeded within actively decomposing carcasses. Therefore, the level of phosphate supersaturation within the tissues, the maintainance of a suitable chemical environment, and the mediation of ionic strength and abundance of inhibitors, were entirely beyond physiological control. Furthermore, since the structure and composition of the soft tissues of decaying organisms is unlikely to be the same as those of living systems (except at the very earliest stages of decay), the presence of organic matrices which are known to be involved in biomineralization cannot be assumed to have participated in the phosphatization of soft tissues postmortem.

Secondly, although postmortem phosphatization may be demonstrated to be substrate specific (see Section 8.2.3), a large number of different organisms (see table 8.1), organs (see Section 4.2.2.1), and an extensive area of the carcasses may be affected. Such chaotic phosphatization contrasts markedly with the very selective and precise mineralization of specific predetermined areas in biomineralizing systems.

Lastly, soft tissues phosphatized postmortem frequently display an enormous degree of variation in the style of their mineralization even within a single organism (see Chapter 4).

This reflects the enormous variations in substrate composition, pH, position relative to the source of phosphorus, and rates of decay (see Sections 8.2.2).

The recognition of these differences provides the rationale behind the proposal of a general theory of postmortem phosphatization which is based largely on *modified* models of biomineralization:

8.3.1 INORGANIC POSTMORTEM PHOSPHATIZATION: MECHANISMS

Postmortem phosphatization may be defined as the precipitation of a phosphatic salt(s) within the soft tissues of a progressively decaying carcass as the direct result of exposure to a source of dissolved phosphorus (usually external). Mineral deposition is entirely beyond physiological control and is dictated exclusively by decay-induced changes in the carcass' chemistry (see Sections 8.2.1 and 8.2.2) and in the chemistry of the external environment. Lattice ion supply is controlled by the electrochemical gradient of each ion, and the length of the diffusion path from source to the mineral forming site. In the simplest of cases (i.e. in the absence of inhibitors), phosphatization will proceed when the solubility product for apatite is exceeded, and will be most extensive closest to the source. This will take place at a rate determined by the degree of supersaturation, and the number and 'reactivity' (extent to which the nucleation activation energy is reduced) of nucleation sites.

As stipulated in Section 8.2.3, the biomolecules of decaying organisms appear to have participated in mineralization either by enclosing a space with a specific chemistry, or by acting as a site for the nucleation of HAP. The processes of postmortem phosphatization can accordingly be divided into two broad groups based on the *major* role of the organic matrices:

1) **PHOSPHATIZATION WITHIN COMPARTMENTS:** It is perhaps surprising that despite the cessation of all physiologically regulated processes (in particular transmembrane ionic pumps) and the likely effects of decay on membrane integrity, many membrane-bound compartments appear to have been able to maintain an independent microenvironment postmortem. This is expressed in the fossil material either by a) the non-

mineralization of the membrane-enclosed space; b) the preferential mineralization of the membrane-enclosed space; or c) a difference in the morphology of crystal microfabrics replacing the enclosed space to that of the adjoining area. There are two principal mechanisms by which spaces enclosed by a biological membrane may maintain an independent chemistry. These are:

i) The presence of inhibitors: Inhibitors, even in trace quantities have the effect of either preventing the direct nucleation and growth of HAP, or of delaying the autocatalytic conversion of ACP to HAP (see Section 3.3.5). Due to the susceptibility of many subcellular bodies to pathological biomineralization (see below), inhibitors were probably an essential component during life. For example, mitochondria contain a high concentration of Ca^{2+} and dissolved phosphorus; T-tubules an abundance of Ca^{2+}; and squamous epithelial cells of the alimentary tract are continually exposed to gastric fluids rich in both dissolved phosphorus and Ca^{2+}. Without inhibitors, these structures would have been in continual risk of phosphatization. The corresponding rarity of *phosphatized* examples of these structures in the soft tissues of fish from the Romualdo Member but their presence as 'ghosts' (e.g. mitochondria [see fig. 4.21] and T-tubules [see fig. 4.24]), suggests that many nucleation inhibitors remained effective and *in situ* postmortem.

In the case of mitochondria, phosphocitrate (Lehninger, 1983), ATP and ADP were probably responsible for inhibiting nucleation during life. The presence of several examples of heavily phosphatized mitochondria in the fossil muscle (see figs. 4.19 and 4.20) however, suggests that the effectiveness of these inhibitors postmortem was reduced. This, I propose, is the result of their exposure to indigenous lysosomal enzymes. As in pathological biomineralization (Smith, 1982), the hydrolysis of phosphocitrate, ATP and ADP would not only permit the precipitation of HAP, but would also encourage phosphatization by locally increasing the level of phosphate supersaturation.

ii) Ion censorship (the selective diffusion of ions across membranes): Although biological membranes are incapable of preserving a compartment's original chemical identity without the aid of physiologically driven ion pumps (Sachs, 1977), a microenvironment

considerably different to that of the surrounding environment may be established through selective ion censorship. This is only possible, as long as the integrity of the bilipid structure remains intact. With the advance of decomposition, the ionic composition of the internal space will eventually equilibrate with that surrounding it.

The efficiency with which dead cells may maintain an independent chemistry (particularly pH and anion levels) can be modelled with synthetic unilamellar vesicles. Many species including small ions (Na^+, K^+, and Cl^-), hydrophobic molecules and small uncharged polar molecules rapidly equilibrate across membranes down their electrochemical gradients (Walter and Gutknecht, 1986). Others however, such as HPO_4^{2-} and PO_4^{3-} take considerably more time to do so due to the repulsion of their high charges by the hydrophobic regions of the bilipid membranes (Mann *et al.*, 1983).

Mann *et al.* (1983) have demonstrated experimentally that through selective ion censorship, bilipid membranes may enclose a space with a pH distinct from that of the surrounding tissue. This relies on a reduction in the difference of pH across the membrane requiring a net influx or efflux of OH^- or H^+. In order to preserve electroneutrality, this demands that there be an equivalent net migration of other similarly charged ions either into or out of the structure. In the case of the decaying fish in the Romualdo Lagoon, in effect, the only ions available to partake in the latter rate-determining step are likely to have been PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^-$. If the pH of the carcass was such that PO_4^{3-} and HPO_4^{2-} were stable (see Section 8.2.1), diffusion across the highly hydrophobic membranes would have been severely impaired or even halted, thereby preventing pH equilibration.

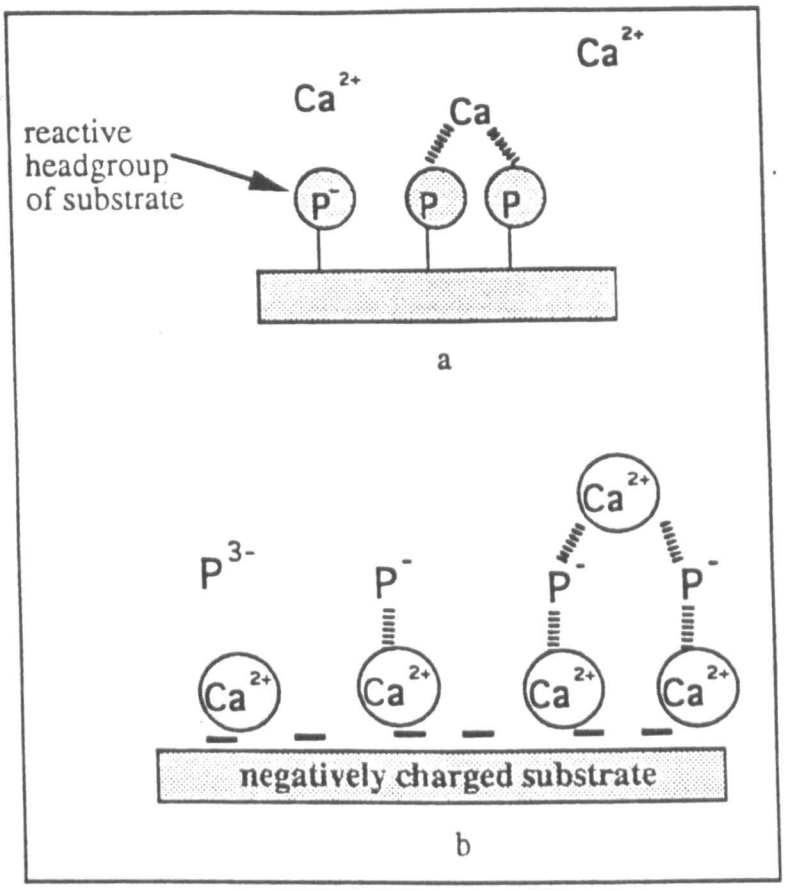
Therefore, due to the selective censorship of certain chemical species, the chemical composition and pH of areas enclosed by membranes may be significantly different to that of the surrounding area. This creates the possibility for differential phosphatization either by a) preventing the efflux of nucleation inhibitors, b) maintaining a different concentration of dissolved phosphorus to the surrounding area, c) maintaining a different pH to that of the surrounding tissue, or d) any combination of these. Which one(s) is responsible for the differential mineralization of specific organelles remains to be tested. However, one can speculate. In the case of mitochondria, phosphatization of the matrix may have been prevented by a combination of: 1) the retention of inhibitors (see above), 2) the

impermeability of the inner membrane of mitochondria to most ions (see Section 6.2.1.2), and 3) the naturally high pH of mitochondria (typically ≈8, Alberts *et al.*, 1989, p352). In contrast, the abundance of phosphatized nuclei may be explained by the high permeability of their membranes and thus their relatively free access to any external source of dissolved phosphorus. The concentration of phosphorus in the nuclear lumen may have been further enriched by the degradation of their phosphorus-rich and relatively volatile chromatin (see Section 6.2.1.2).

It is possible in cases where the diffusion of dissolved phosphorus into membrane-enclosed spaces was extremely rapid, that the sudden increase in phosphate saturation would have favoured the precipitation of ACP (Boskey and Posner, 1976). Similarly, ACP is likely to have been the stable phase in organelles containing nucleation inhibitors. In contrast, in those organelles which permitted only minimal transmembrane diffusion of dissolved phosphorus, and/or provided an alkaline microenvironment in which PO_4^{3-} and HPO_4^{2-} were stable, only low levels of intra-organelle supersaturation would result, leading to the precipitation of HAP (Boskey and Posner, 1976).

Similar minor variations in the pH, degree of supersaturation, and presence of nucleation inhibitors, have been invoked by a number of authors to explain the restriction of sedimentary authigenic apatite to minute microenvironments (e.g. Weaver and Wampler, 1972; Burnett, 1977; Baturin and Bezrukov, 1979; Balson, 1980).

2) **HETEROGENEOUS PHOSPHATIZATION:** Undoubtedly, much of the substrate specificity displayed by the soft tissues of fish from the Romualdo Member reflects variations in the organic matrices' ability to reduce the nucleation activation energies of HAP and therefore stimulate phosphatization. This may be achieved either by the substrate a) possessing a 'reactive head group' onto which apatite can nucleate (text fig. 8.9a), or b) by the substrate having an electrostatic charge to which dissolved phosphorus and Ca^{2+} are attracted (text fig. 8.9b). Similar processes have been proposed for the nucleation of minerals in living systems (see Termine, 1980, fig. 4; Mann, 1983).



Text figure 8.9: Mechanisms by which organic matrices may induce nucleation. a) Nucleation onto "reactive head groups": calcium ions are attracted to and react with headgroups to initiate nucleation. b) Electrostatically-driven nucleation: calcium ions are attracted to the negatively charged substrate and form a reactive surface onto which phosphate ions and then more calcium ions are attracted (based on Termine, 1980; Mann, 1983).

These models are discussed separately below in terms of decomposing tissues:

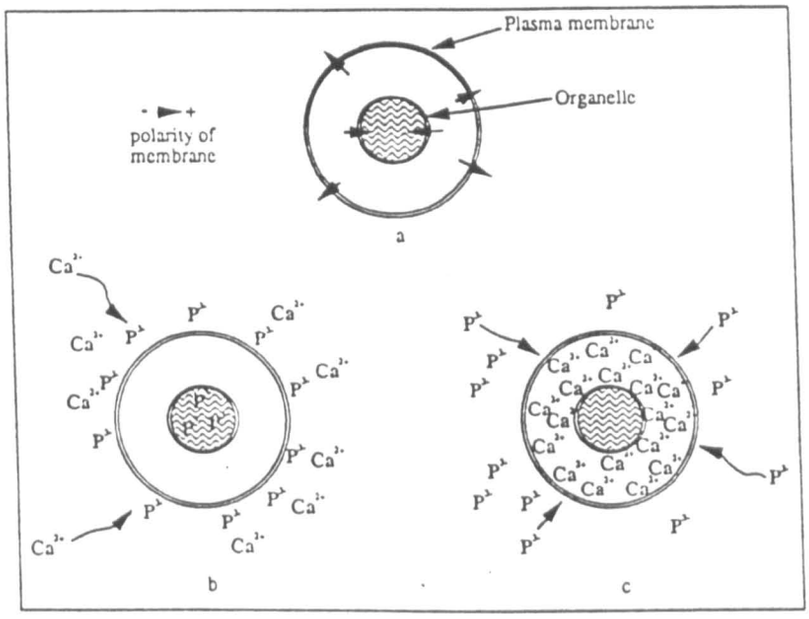
a) Reactive head groups: Bilipid membranes are chemically complex structures composed of thousands of different types of lipids, proteins and carbohydrates, the exact combination of which is different for different cells and types of membranes (see Alberts *et al.*, 1989, pp275-340). Variations in the ability of these molecules to induce the nucleation of HAP is reflected in the preservation of plasma membranes in some cells as internal moulds, whereas the external surface of their organelles remain entirely unmineralized despite being exposed to the same chemical environment (see figs. 4.50, 4.51). Unfortunately, although the composition of the outside and inside faces of individual membranes are known to be different (see Alberts *et al.*, 1989, pp275-340), it is difficult to identify with any degree of certainty which molecule(s) acted as the site(s) of nucleation in the fossil material. In biomineralizing systems, phosphoproteins, glycoproteins, calcium-

acid phospholipid-phosphate complexes, and proteolipids, all of which are common components of plasma membranes, have been proposed as probable substrates for the nucleation of apatite (see Williams, 1984). However, only in the case of skeletal muscle and dermis (see below), and some fossil stomach wall epithelial cells (see fig. 4.35) has the substrate been tentatively identified. In the case of the stomach wall epithelial cells, apatite was deposited as a thin internal mould which extends into the microvilli. Actin filaments are abundant in the cortex of most cells (a thin shape-controlling band just beneath the plasma membrane) and are especially abundant within microvilli. I therefore propose that these actin filaments provided the substrate for nucleation. However, since actin filaments are common to most cells, and mineralization was extremely cell-specific in the fish of the Romualdo Member, it would appear that the presence of this molecule is not sufficient to induce mineralization in all cases. More likely, different substrates (but with a similar structure or 'reactive' head group) were responsible for nucleating HAP according to the prevailing chemical environment. That is, under extremely high levels of supersaturation most substrates are capable of inducing nucleation, whereas when the level of supersaturation is reduced, only specific matrices which are particularly well suited to nucleating apatite (e.g. actin) will do so.

b) Electrostatic charges: In those cells which have either a) the organelles preserved as external moulds and the plasma membrane preserved as an internal mould, or b) the organelles preserved as internal moulds and the plasma membrane preserved as an external mould (see fig. 8.3), it is *possible* that the electrochemical charge of the membranes played the dominant role in stimulating the precipitation of apatite.

All living cells and organelles create an electrical potential across their membranes as a means of stabilizing their volume and of transporting ions. At the point of death this will be much reduced due to the 'leakage' of ions down their electrochemical gradients, but will stabilize at -20mV to -200mV (Alberts *et al.*, 1989, p316). The polarity of organelles is opposite to that of the cell's plasma membrane so that the two facing monolayers of each one's membrane have the same polarity (see text fig. 8.10a). Therefore, when introduced to a solution supersaturated with respect to apatite, depending on which side of the membranes

HAP is stable, either Ca^{2+} ions or the negatively charged phosphate and orthophosphate ions will be attracted to them. These will form a layer on which the other ion may then nucleate (text fig. 8. 10b and c).



Text figure 8.10: Electrostatically-driven nucleation in decomposing tissues. a) An electrical charge exists across all biological membranes; that of the outer surface of organelles is the same polarity as the inner surface of the plasma membrane. b) HAP is stable extracellularly and within the organelles. Dissolved phosphorus is attracted to the outer surface of the plasma membrane and inner surface of the organelle's membrane. To these, calcium is subsequently attracted thereby initiating nucleation. c) HAP is stable in the cytosol. Calcium ions are attracted to the inner surface of the plasma membrane and outer face of the organelles where later attraction of dissolved phosphorus initiates nucleation.

Perhaps the most convincing illustration of the involvement of electrostatic charges in encouraging the nucleation of apatite is given by fossil mitochondria. When active, mitochondria have a relatively high membrane potential (typically ≈220mV, Alberts *et al.*, 1989, p352) as a consequence of the manufacture of ATP. The rate at which this charge is dissipated postmortem by the leakage of H^+ into the matrix is uncertain. However, due to the relative impermeability of the inner membrane of mitochondria, I propose that a significant positive charge will remain postmortem across their membranes, thereby attracting ions in solution (i.e. phosphate, orthophosphate, and calcium). To these, other ions will be electrostatically attracted and nucleation will have initiated. This model is supported by the fossil mitochondria identified in the striated muscle of fish from the Romualdo Member, and in particular by one identified in TEM (fig. 8.4). In the latter, the

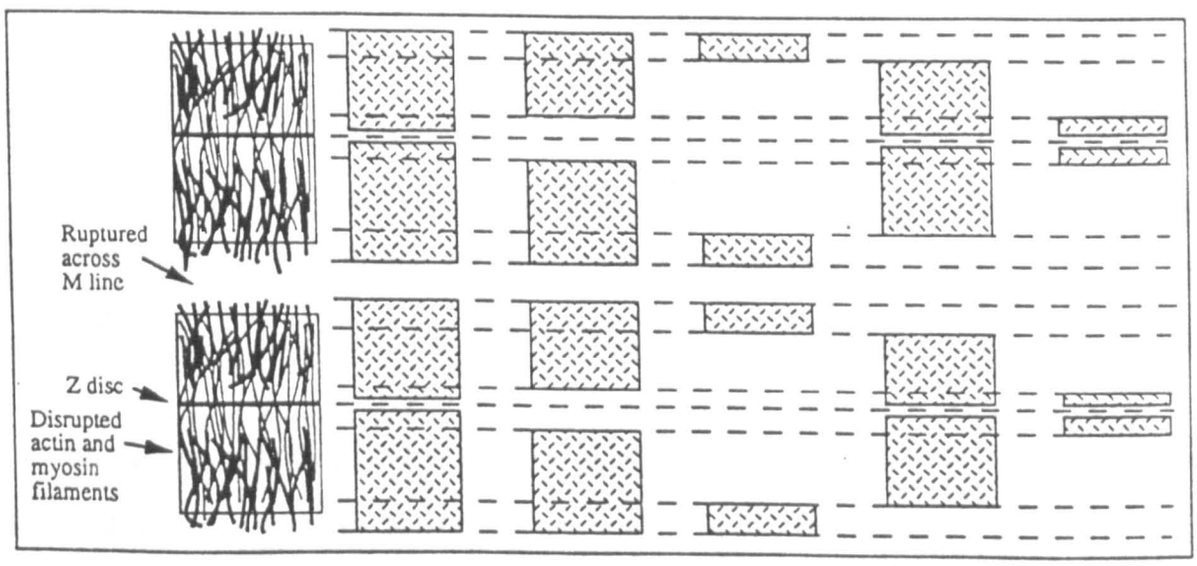
intermembrane space is reproduced with absolute precision. Since the outer- and inner-membranes have very different roles to perform, they are likely to have had somewhat different compositions and therefore a substrate specific mechanism of nucleation is unlikely. In contrast, the polarity of the juxtaposed faces of the two membranes are the same implying an electrostatically-driven mechanism of nucleation. A similar mechanism has been proposed by Termine (1980, fig. 4) for the mineralization of dentin.

The division of postmortem phosphatization into two distinct groups (see above) is somewhat artificial. For example, only when leakage of ions across the plasma membrane has established a chemistry within the cell conducive to the precipitation of apatite will specific substrates be capable of inducing nucleation; the two are not mutually exclusive. To demonstrate the difficulties involved in establishing the role(s) of organic matrices in the fossilization of soft tissues, the nucleation sites of HAP in two common tissues are discussed below:

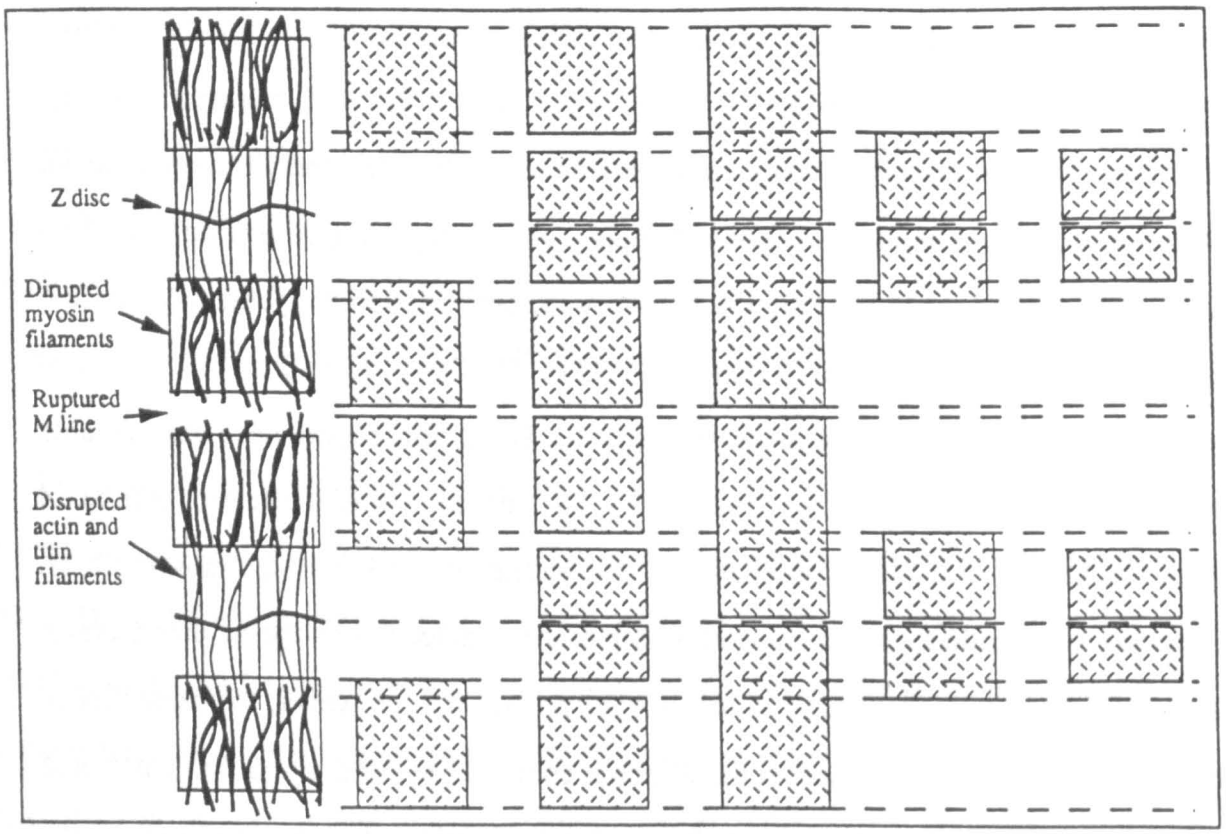
i) Striated muscle: The extremely ordered structure and considerably researched composition of biomolecules in striated muscle, makes this tissue ideally suited to identifying some of the substrates responsible for inducing HAP nucleation postmortem. For example, taphonomic experiments (see Chapter 6) indicate that at the time of fossilization, with the exception of myosin filaments, all of the major components of striated muscle were *relatively* intact (i.e. in TEM investigations the components retain an appearance comparable to that of pristine muscle). Therefore, since fossil Z-discs have never been observed, I conclude that α-actin (the principle component of Z-discs) did not act as a nucleation site.

Which matrices were, and which were not involved in the nucleation of apatite in the sarcomeres themselves (i.e. the actinomysin complexes) however, is less clear. Schultze (1989), mistaking taphonomic banding for differential mineralization (see Section 6.2.1.2) proposed three alternative nucleation sites (see text fig. 6.3a-c). Even with a greater understanding of the taphonomy of striated muscle (see Chapter 6), the number of combinations of sites capable of producing banding patterns *similar* to those of the fossil muscle are great (text figs. 8.11 and 8.12). However, in some fossil muscle fibres in which

the actin and myosin filaments have become completely disassociated (see text fig. 6.1: 3b), phosphatization is restricted almost entirely to the region of the actin filaments (see fig. 6.19). This precludes myosin as being the substrate responsible for mineralization in favour of either actin, titin, nebulin, the troponin complex, or tropomyosin (see text fig. 6.2). This is consistent with the inability of myosin to induce the precipitation of apatite *in vitro* from metastable phosphate solutions (Glimcher, 1959). Similarly, Glimcher (1959) found tropomyosin to be incapable of nucleating apatite. Since titin experiences considerable damage during rigor mortis (see Section 6.2.1.2), nebulin and/or the troponin/actin complexes are the most likely candidates for nucleating apatite in striated muscle fibres. Unfortunately, the intimate structural relationship of these molecules to one another makes it impossible from the fossil material to identify the precise site. However, the capability of troponin (or at least a polypeptide in the troponin complex) to 'bind' up to four Ca^{2+}, and the availability of 'reactive' phosphorus head groups in actin (when not inhibited by the troponin complex, see Section 6.3.3), suggests that these two matrices are more likely candidates than the relatively inert nebulin molecules. Extrapolating from the already suspected role of actin in the phosphatization of epithelial cells (see above), actin was probably the nucleation site of HAP in muscle.



Text figure 8.11: Differential phosphatization of sarcomeres (running N-S) following rupture of their M-lines (refer to text fig. 6.1: 2b). Taphonomic banding *similar* to that of fossil striated muscle (see Section 6.2.1.2) may be produced by the selective phosphatization of a number of "sites" in the sarcomeres (shading indicates mineralization). See text for details.



Text figure 8.12: Differential phosphatization of sarcomeres (running N-S) following extreme tension and rupture of their M-lines (refer to text fig. 6.1: 3a). Taphonomic banding *similar* to that of fossil striated muscle (see Section 6.2.1.2) may be produced by the selective phosphatization of a number of "sites" in the sarcomeres (shading indicates mineralization). See text for details.

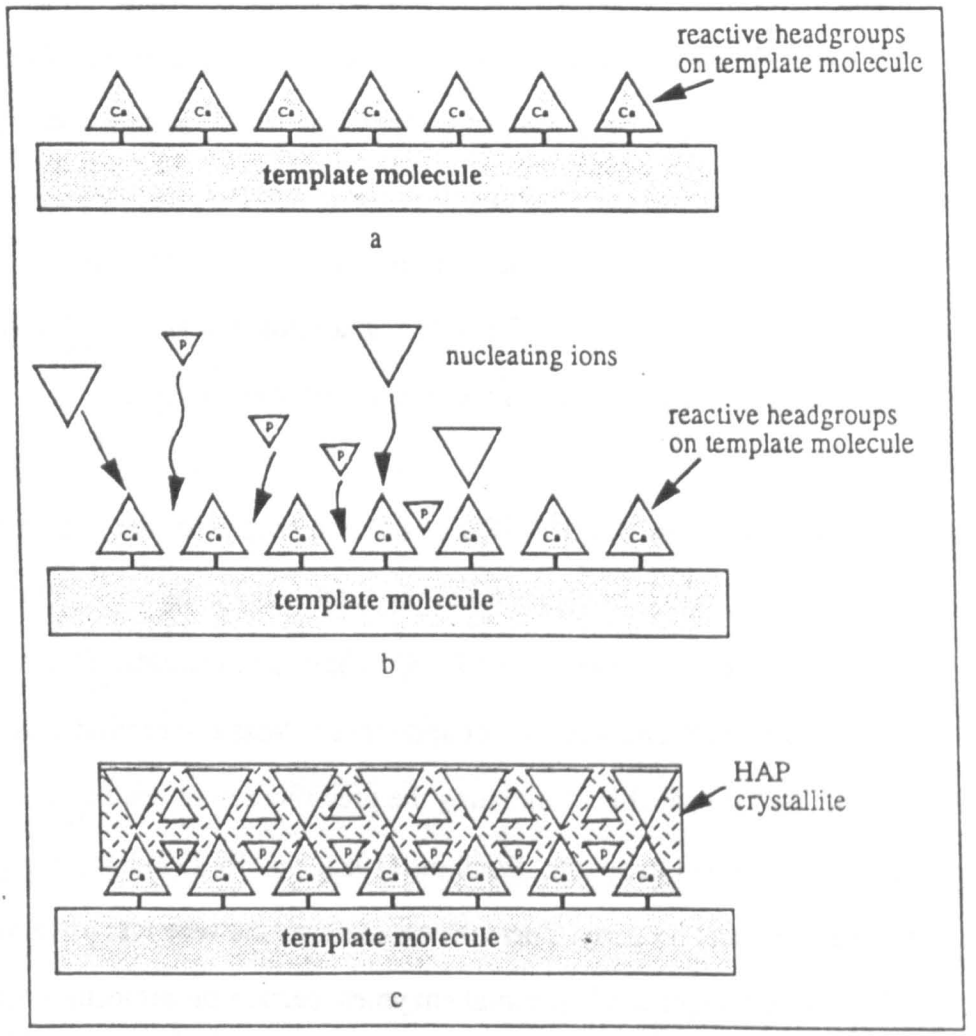
Since the tissues had experienced approximately 55hrs of decomposition prior to mineralization (see Chapter 6), it must be stressed that although actin is tentatively proposed as the nucleation site, at the present level of understanding it is unrealistic to name which reactive groups (or regions) on this molecule were responsible. Indeed, new and very 'reactive' sites are likely to have been created during the initial stages of decay which are entirely different to those present on the original molecule. Smith (1982, p264) regarding pathological biomineralization, similarly concluded that debris resulting from the hydrolysis of cell components created new sites for heterogeneous nucleation.

The skeletal muscle of fish from the Romualdo Member provides another example of the variable ability of organic matrices to induce the nucleation of HAP. Frequently, the sarcomeres of muscle fibres are replaced by microspheres (see fig. 4.13) whereas the connective fibrils linking them to the myoseptum are pseudomorphed by microgranular apatite (see fig. 4.14). Since both the sarcomeres and connective fibrils were presumably

exposed to identical chemical conditions, it would appear likely that either a) the collagen filaments composing the connective fibrils were more capable of nucleating HAP crystallites than the actin filaments of the sarcomeres, or b) that at the pH and level of phosphate supersaturation to which the fibre was exposed, HAP was stable at both sites but was prevented from precipitating in the sarcomeres due to the presence of nucleation inhibitors (presumably ATP and ADP). In either case, it is interesting to note that the collagen filaments display periodic thickenings (see fig. 4.14) on a minute scale (<200nm). This, I propose reflects the nucleation of HAP crystallites only at *very* specific sites.

ii) Recalcitrant biomolecules: Smith (1982, p264) has proposed that the destruction of inhibitors in living systems by lysosomal enzymes may induce pathological biomineralization. Similarly, Glimcher *et al.* (1957, p865) have demonstrated experimentally that once inhibitors are removed, all forms of collagen (even those not normally associated with mineralization *in vivo*) are capable of initiating the precipitation of apatite *in vitro* from solutions metastable with respect to apatite. Wadkins (1981, pp276-277) has demonstrated bovine aorta to have an identical property. There therefore exists the very real possibility that once freed of inhibitors by indigenous lysosomal enzymes, certain biomolecules known to be susceptible to biomineralization will nucleate apatite in decaying carcasses. In order to retain the structure and composition of these substrates that was essential to their mineralization in the living systems, this may only be possible with recalcitrant biomolecules (e.g. collagen, see Section 6.2.1.2).

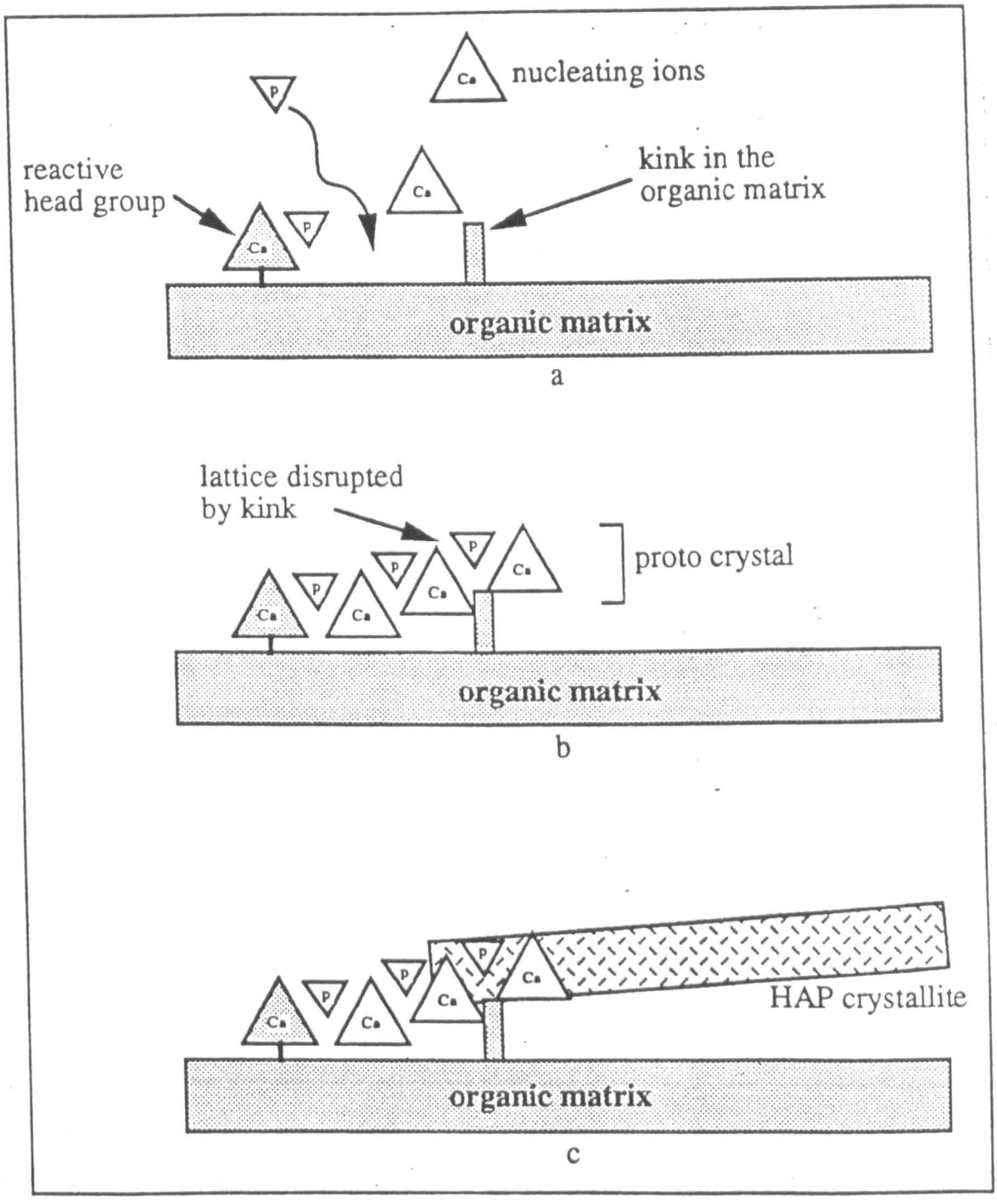
The spatially organised crystallites replacing the collagenous plasma membrane tonofibrils of fish dermis from the Romualdo Member (see figs. 4.3, 4.4) demonstrate that certain recalcitrant substrates did retain their capability for ordered nucleation postmortem. Only on a 'pre-conditioned' and highly organised framework would such mineralization be possible. Indeed, material from specimens of fish dermis displaying a preferential alignment of crystallites are indistinguishable from that of fish bone figured by Glimcher (1959, figs. 9 and 10). The rarity of soft tissues displaying this level of organisation suggests that the stringent conditions required were met only very rarely; the process is extremely matrix-specific.



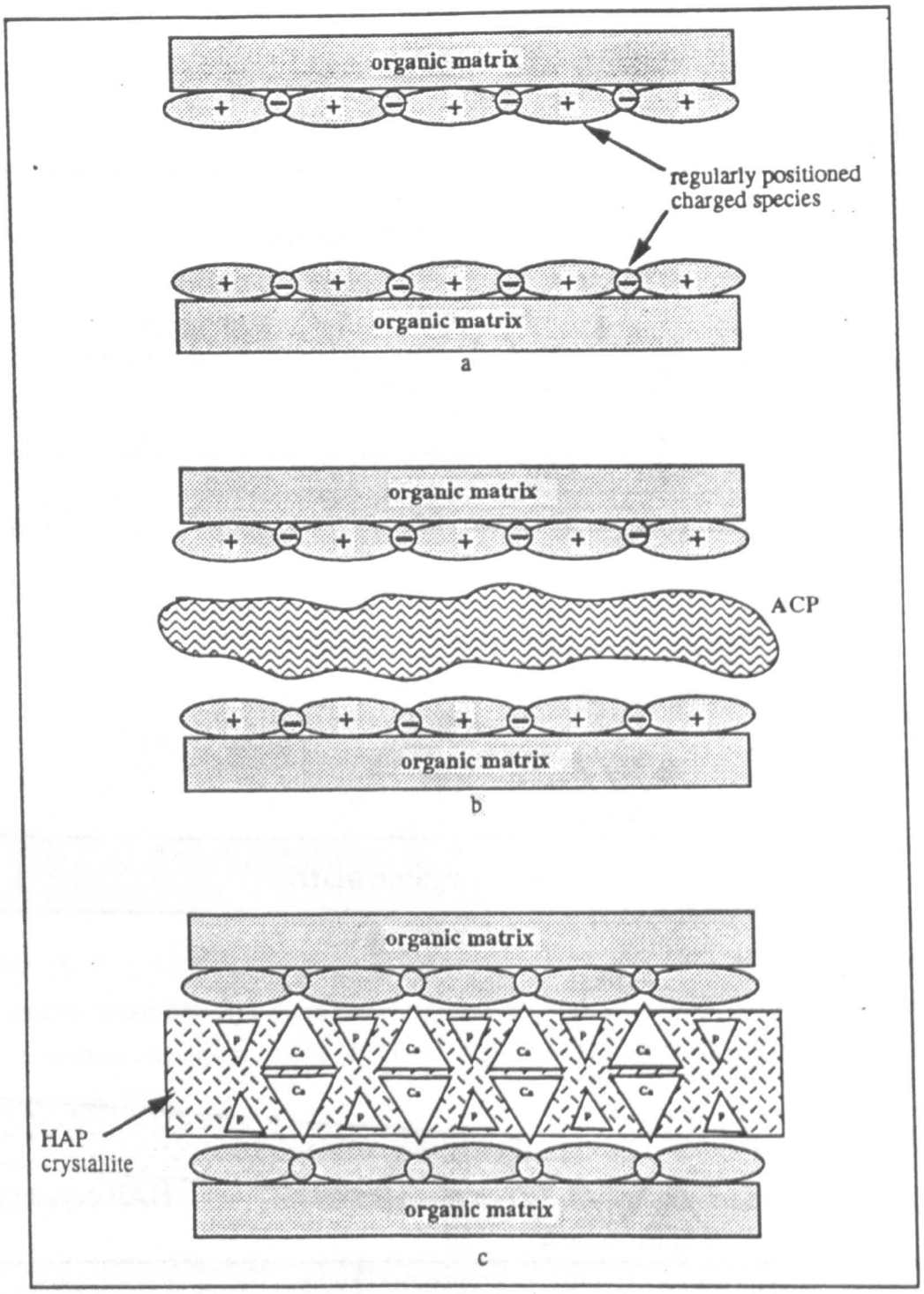
Text figure 8.13: Spatially organised crystallites through epitaxial growth. a) Template molecule containing reactive headgroups whose spacing exactly matches that of the HAP lattice. b) Calcium and phosphate ions nucleate onto template molecule. c) HAP crystallite develops which is aligned parallel to the substrate (after Mann, 1983).

According to Mann (1983), such ordered nucleation in living systems may be achieved in three principle ways. These are: a) by crystallites nucleating epitaxially onto a specific template molecule by atomic matching of the lattice ions (text fig. 8.13); b) by the energetically favoured nucleation of crystallites at points of matrix initiated defects (text fig. 8.14); and c) through the interaction of phosphate and calcium ions with oppositely charged species of the organic framework during the transformation of ACP to HAP (text fig. 8.15). How applicable these models are to the situation described in the decaying carcasses of fish from the Romualdo Member is uncertain. Clearly, there is a necessity for further research

regarding the relative stability of certain molecules postmortem and their capability of inducing mineralization.



Text figure 8.14: Spatially organised crystallites due to matrix-initiated defects. a) Organic matrix with a single reactive headgroup to which calcium and phosphate ions are attracted. b) A kink in the substrate causes a disruption in the lattice of the protocrystal. c) Nucleation is favoured at the point of disruption due to the reduction in nucleation activation energy there (after Mann, 1983).



Text figure 8.15: Spatially organised crystallites as the result of the reorganisation of calcium and phosphate ions. a) A complex organic framework with regularly spaced charged species. b) ACP is initially precipitated. c) During the transformation of ACP to HAP, the ions are reorganised under the influence of the organic matrix in such a manner that the crystallite develops a specific orientation (after Mann, 1983).

8.3.2 SUMMARY

Organic substrates may be involved either indirectly or directly in inorganic postmortem phosphatization. In the former case, they provide a cellular or subcellular microenvironment whose chemistry is maintained different to that of the surrounding tissues either by the retention of nucleation inhibitors (see Section 3.3.5) or by selectively censoring the diffusion of ions into and out of the membrane-bound space. Alternatively, organic substrates may be directly involved in mineralization by providing a specific 'reactive' site for the nucleation of HAP (e.g. actin and collagen) or a charged surface to which the Ca^{2+} and phosphate ions are attracted.

It is clear that the processes of postmortem phosphatization are closely related to those of biomineralization. The lack of physiological control over mineralization after death, and the importance of lysosomal enzymes in a) accelerating the rate at which dissolved phosphorus and Ca^{2+} may invade the tissues, b) destroying nucleation inhibitors, c) increasing the degree of calcium phosphate saturation (through the hydrolysis of ATP, ADP and other phosphorus-rich organomolecules), and d) creating new 'reactive' nucleation sites, implies a particularly close affiliation with pathological (abnormal) biomineralization. In pathological biomineralization, mineralization is accompanied (or initiated) by cell necrosis (see Smith, 1982). A period of decay is similarly an essential prerequisite of postmortem phosphatization. There is however, a delicate balance between establishing conditions favourable to phosphatization, and preventing the excessive loss of information and the ability of membranes to enclose independent microenvironments.

Taphonomic and textural similarities shared by the inorganically mineralized muscle fibres of several deposits (e.g. the Romualdo Member [see Chapter 4], the Glencartholm Volcanic Beds [see Section 5.2.13], and the Cleveland Shale [see Section 5.2.14]) suggests that this balance, and the processes of phosphatization as a whole, were in all cases essentially the same.

8.4 CONCLUSIONS

1) A source of phosphorus (whether internal or external to the carcass) is not in itself adequate to induce the phosphatization of decomposing soft tissues; mineralization is dictated by a complex interplay of a number of variables including the concentration of phosphorus in the tissues, proximity to the source of dissolved phosphorus, rate of decay relative to the timing of mineralization, and the pH and chemical composition of the decay-induced microenvironment. Differences in any one of these parameters can result in entire groups of organisms, individual carcasses, organs, cells, or organelles not being phosphatized.

2) Certain decomposing organisms (especially fish, squid, and bivalves) are capable of maintaining an independent and favourable chemistry to that of the external environment, and thus provide a suitable site for the precipitation of phosphatic phases.

3) Although sharing similarities with pathological biomineralization, differences in the timing, level of physiological control, and extent of mineralization, mean that postmortem phosphatization cannot be adequately described in terms of any current theory of biomineralization.

4) Organic matrices participated in postmortem phosphatization either: i) indirectly, by enclosing a space, the chemistry of which was maintained independent to that of the surrounding tissues by means of passive ion censorship and/or the presence of inhibitors; or ii) directly, by providing a substrate capable of acting as a site for the nucleation of HAP.

5) A period of decay is an essential prerequisite for inorganic postmortem phosphatization. Indigenous lysosomal enzymes i) accelerate the rate at which dissolved phosphorus and calcium may invade the tissues, ii) create new reactive substrates for the nucleation of HAP, and iii) destroy nucleation inhibitors.

Microbes are important in creating a favourable decay-induced microenvironment for the release of phosphorus from the surrounding sediment (see Section 7.2.3) and for the precipitation of phosphates within the carcass, but are not otherwise involved in *inorganic* postmortem mineralization.

CHAPTER 9
SUMMARY

9.1 SUMMARY

Despite the enormous palaeobiological importance of phosphatized soft tissues, surprisingly little is known of the processes involved in their genesis (particularly at the tissue level), the source of their phosphorus, or the timing of their mineralization. This thesis presents new data regarding all three of these aspects with particular emphasis on the phosphatized soft tissues of the Lower Cretaceous Romualdo Member biota. In addition, I have: described the preservational styles of phosphatized soft tissues from nine separate biotas (see Chapter 5); documented the initial stages of decay of Recent soft tissues at the subcellular level across a wide spectrum of environmental conditions (see Chapter 6); and, catalogued in detail the abundance, distribution (stratigraphical, lateral, and taxonomic), diversity, microfabrics and preservational styles of phosphatized soft tissues in the Romualdo Member biota (see Chapters 2, 3 and 4). This thesis represents one of the most detailed and extensive investigations of phosphatized soft tissues thus far performed, and may therefore be of some use as a reference "standard" in the examination of other phosphatized biotas.

The main conclusions of this thesis are summarized below under 7 key headings (Sections 9.1.1-9.1.7):

9.1.1 THE DISTRIBUTION OF PHOSPHATIZED SOFT TISSUES IN THE ARARIPE BASIN

Phosphatized soft tissues occur both in the Nova Olinda Member of the Crato Formation and in the Romualdo Member. In the Romualdo Member, phosphatized soft tissues are extremely abundant and occur across the entire extent of its outcrop. They are not restricted to any specific concretionary horizon but are most abundant in type 2 concretions (see Chapter 2), where up to 10% of the fish contain phosphatized soft tissues. The absence of phosphatized soft tissues in the other fish suggests phosphatization in the Romualdo Member to have been spasmodic.

In the Romualdo Member, phosphatized soft tissues occur in pterosaurs, fish and crustaceans. Regarding the fish, phosphatized soft tissues occur frequently in *Rhacolepis* sp., *Notelops* sp., *Brannerion* sp., *Tharrias* sp., and *Rhinobatos* sp. They have also been recorded from *Vinctifer* sp., *Cladocyclus* sp., and *Tribodus* sp. Their apparent absence in other fish taxa is probably *partly* a consequence of the scarcity of specimens of these fish.

Except for some decapod shrimps at the far western edge of the Chapada, phosphatized soft tissues in invertebrates *appear* to occur only when associated with vertebrates. This *may* reflect their incapability of independently creating a decay-induced microenvironment favourable to the precipitation of apatite, but is more likely a consequence of collector bias.

It is clear from an examination of the alimentary tracts of fish from the Romualdo Member, that certain vertebrates have enormous potential for preserving otherwise rarely preserved invertebrates in exceptional detail and at concentrations well above those encountered in the sediment.

9.1.2 THE PALAEOENVIRONMENT OF THE ROMUALDO MEMBER

The shales of the Romualdo Member were deposited in a relatively shallow, warm lagoon with a permanent but restricted connection to the Atlantic. Large volumes of freshwater were periodically flushed in by deltas, which together with the lagoon's restricted nature, created salinity fluctuations in the surface waters and excluded many of the typical marine organisms of the Cretaceous from becoming established. A persistent halocline separated the dysoxic, hypersaline bottom waters of the Romualdo Lagoon from its well oxygenated surface waters. The upper 20cm+ of sediment was soupy and covered in microbial mats. Sedimentation rates were high (2.6 cm/yr) but probably episodic. For much of the time, the soupy substrate was sparsely populated by small bivalves, gastropods and ostracodes tolerant to the dysoxic, hypersaline conditions. The fish do not appear to have been capable of venturing into the hypolimnion. In the shallows at the margins of the Romualdo Lagoon, stromatolites and bioclastic limestones developed.

These background conditions were periodically interrupted by storms which forced the mixing of the water column and induced repeated fish mass mortality events. In the

sediment, concretions (or at least adipocere precursors) developed around fish carcasses within a matter of weeks of the fishes' death.

9.1.3 THE CRYSTAL MICROFABRICS OF PHOSPHATIZED SOFT TISSUES

All phosphatized soft tissues are preserved by one or more of three groups of microfabrics. These are: 1) mineralized microbes; 2) inorganic precipitates which have undergone recrystallization (i..e microspheres); and, 3) inorganic crystallites which nucleated directly onto the organic substrates from solution (i.e. microgranular apatite). These microfabrics may preserve soft tissues in a number of different ways (i.e. coatings, replacements, etc, see Section 9.1.4).

The distinction between microbial and inorganic microfabrics is genetic. However, since both microbial and inorganic microfabrics may occur within individual fossils, the geochemical conditions required for their production must overlap. In cases of microbial mineralization, the soft tissues themselves are not mineralized, but rather the infesting microbes are. The phosphorus for the mineralization of the microbes may be derived from the tissues they are metabolizing and/or from an external source. Alternatively, in cases of inorganic mineralization the soft tissues themselves are pseudomorphed by apatite crystallites or crystal aggregates identical to those which precipitate *in vitro* from supersaturated solutions by "simple" inorganic precipitation. The phosphorus for such mineralization appears to be derived from beyond the tissue undergoing mineralization (and may therefore be from elsewhere in the organism).

Each of the three distinctive microfabrics replicates soft tissues with a predictable level of precision. Microbes rarely preserve details finer than the tissue's gross morphology; inorganic microspheres are capable of pseudomorphing subcellular structures; and granular apatite may fossilize macromolecular details. Soft tissues replaced by microgranular apatite therefore offer palaeontologists their greatest opportunity to examine the biology, physiology, and evolutionary relationships of extinct organisms.

9.1.4 THE PRESERVATIONAL STYLES (OR MECHANISMS OF MINERALIZATION) OF PHOSPHATIZED SOFT TISSUES

All phosphatized soft tissues may be expressed in terms of one or more of three preservational styles. These are: 1) replacement by the direct precipitation of apatite onto the constituent biomolecules of the tissue; 2) the coating of relatively decay-resistant biomolecules (e.g. arthropod cuticle) by apatite; and, 3) microbial infestation, where microorganisms either pseudomorph or coat the tissues and are themselves the sites of apatite nucleation. Microbial infestations are by far the most common preservational style/mechanism of mineralization. Each preservational style corresponds to a distinct but genetically related process of mineralization.

Each of the three preservational styles preserves a predictable level of detail. This is a reflection of the similarities in rates of tissue decay and microbe infestation, phosphate diffusion into the tissues, and the chemical composition of the organisms. Microbial infestations rarely preserve more than cellular details; inorganic coatings most frequently preserve only whole organs; and, inorganic replacements most characteristically preserve sub-cellular details (but may replicate macromolecular details).

In general, comparable tissues within closely related taxa from different deposits have similar preservational styles. For example, the preservation of soft tissues in arthropods is dominated by microbial infestations and inorganic coatings; coleoids by microbial infestations; and fish by inorganic replacements and microbial infestations. The soft tissues of different groups of organisms are therefore preserved at a predictable level of detail. Arthropods are characterised by the preservation of whole organs, whilst subcellular details are the norm in fish.

9.1.5 MICROTAPHONOMY AS A PALAEONTOLOGICAL TOOL

Microtaphonomic studies are crucial to an understanding of the processes and timing of soft tissue fossilization; they permit the quantity of information obscured, destroyed, and/or not preserved by the mineralizing process to be established. When decomposed in the absence of microbes, the sequence and timing of appearance of taphostructures in Recent

skeletal muscle and other soft tissues are predictable over a wide range of environmental conditions, and are reproducible with remarkable accuracy. In the absence of microbes, the rate of decomposition is dictated largely by the relative activity of indigenous lysosomal enzymes, the severity of rigor mortis, and the level of osmotic stress.

The reproduction in Recent striated fish muscle of distinctive taphostructures that are preserved in fossil fish muscle from the Romualdo Member, suggests phosphatization to have taken place within 55 hrs of death, and to have occurred in hypersaline waters. Fossil fish muscle from the Cordillera de Domeyko, the Glencartholm Volcanic Beds, and the Cleveland shale displays a comparable level of decay to that of fish muscle from the Romualdo Member. This suggests phosphatization to have occurred in all of the fish of these three deposits at *roughly* the same time postmortem.

High resolution TEM examinations of Recent fish muscle indicates that at the time of phosphatization of the Romualdo Member fish, many of their constituent biomolecules would have been intact or at least partially intact. Membranes would have been coherent, actin and myosin filaments still discernible (although somewhat disorganised), and collagen would still have retained its characteristic banding pattern. However, chromatin and other "volatile" biomolecules would have suffered extensive degradation prior to the phosphatizing event.

All fossil phosphatized soft tissues display some evidence of decay. However, differences in the preservational style (and therefore mechanism of mineralization) and/or source of phosphorus for different organisms even within the same deposit, suggests the timing of soft tissue phosphatization to be taxonomically determined.

9.1.6 THE SOURCE OF PHOSPHORUS

For thermodynamic reasons, soft tissues may only be phosphatized in suitable microenvironments (see Section 9.1.7) when the concentration of phosphorus has been concentrated (either organically or inorganically) above an as yet undetermined threshold.

Phosphatization of soft tissues in the Romualdo Member was spasmodic and restricted only to those organisms that did not sink below the upper few centimetres of the soupy substrate. Gradients in the density of mineralization of soft tissues in fishes from the

Romualdo Member suggest the dominant source of phosphorus to have been external to the carcasses. This was almost certainly ultimately derived (and released by microbes) from organic detritus. However, phosphate fluxes in comparable Recent environmental settings suggest that without a mechanism of concentrating the phosphorus released from sedimented organics, the flux of phosphorus released into the Romualdo Lagoon from the sediment would not have been great enough to induce the phosphatization of soft tissues. Phosphorus was therefore probably concentrated in the oxygenated upper few centimetres of the sediment by adsorption onto clays and ferric hydroxides. This phosphorus would then be released *en masse* around decomposing organisms by the growth of their reducing decay-microenvironments.

Taphonomic and palaeoenvironmental characteristics common between the Romualdo Member and many other Lagerstätten containing phosphatized soft tissues, suggest that they had a similar source of phosphorus, and a similar mechanism of concentrating the phosphorus in the sediment and releasing it to the carcasses. Such deposits are characterised by the early diagenetic growth of protective $CaCO_3$ concretions, a high concentration of sedimentary organics, and deposition within a restricted environment. The process of soft tissue phosphatization may be considered to be an unusual end-member of phosphogenesis.

Some soft tissues were phosphatized by an internal source of phosphorus (i.e. phosphorus derived from within the carcass itself). Such cases are rare; in most cases at least some phosphorus must be derived from an external source. The most important internal sources of phosphorus are the organism's own gastric contents and organically-bound phosphorus released by microbes from the organism's own tissues.

Internal sources of phosphorus are most characteristic of crustaceans and bivalves with phosphatic gill supports. Occurrences of soft tissues mineralized by an internal source of phosphorus are not particularly characteristic of any environment, although depositional settings prone to catastrophic burial or the growth of algal mats may be favourable due to the the promotion of restricted anoxic conditions.

9.1.7 THE MECHANISM OF PHOSPHATIZATION

An available source of phosphorus is not in itself adequate to induce the phosphatization of soft tissues in decomposing organisms; mineralization is dictated by a complex interplay of a number of variables. These include: a) the concentration of phosphorus in the tissues, b) proximity to the source of dissolved phosphorus, c) rate of decay relative to the timing of phosphatization, and d) the pH and chemical composition of the decay-induced microenvironment. Differences in any one of these parameters can result in entire groups of organisms, individual carcasses, organs, cells, or organelles not being phosphatized. Certain decomposing organisms (e.g. fish, squid, and bivalves) are intrinsically suited to postmortem phosphatization.

A short period of decay is an essential prerequisite for inorganic postmortem phosphatization. Decay creates a favourable decay-induced microenvironment for the precipitation of apatite, and releases indigenous lysosomal enzymes which: i) accelerate the rate at which dissolved phosphorus and calcium invade the tissues from external sources, ii) create new reactive substrates for the nucleation of HAP through their destructive action, and iii) destroy nucleation inhibitors.

The organic matrices of the decomposing fossil organisms participated in postmortem phosphatization either: i) indirectly by enclosing a space, the chemistry of which was maintained independent to that of the surrounding tissues by means of passive ion censorship and/or the presence of inhibitors; or ii) directly, by providing a substrate capable of acting as a site for the nucleation of HAP. Postmortem phosphatization therefore shares a number of similarities with pathological biomineralization. However, differences in the timing and level of mineralization, and the level of physiological control, mean that the postmortem phosphatization of soft tissues cannot be adequately described in terms of any current theory of biomineralization.

9.2 FUTURE RESEARCH

This thesis has highlighted three related areas of research which remain to be investigated and which promise to be extremely informative. These are:

1) Examination of phosphatized soft tissues in other lagerstätten: It would now be extremely informative to make a detailed sedimentological and palaeoenvironmental examination of a number of other deposits containing phosphatized soft tissues. In particular, a *detailed* re-examination of the microfabrics, preservational styles, and distribution (depositional, and taxonomic) of phosphatized soft tissues in the Cleveland Shale (Devonian), the Glencartholm Volcanic Beds (Carboniferous), the Solnhofen Limestone (Jurassic), Christian Malford (Jurassic), and the Portland Roach (Jurassic) is long overdue; and it would be interesting to examine material from the Manse Burn Formation. In particular, such an investigation would a) permit the conclusions reached in this thesis to be tested; b) permit the extent of the organism- and tissue-specificity of phosphatization to be examined; and, c) allow the identification of the environmental controls on phosphatization.

2) Actualistic experimentation: It would be logical to complement Briggs and Kears (1993a) experiments by attempting to phosphatize soft tissues in the laboratory with emphasis on changes in the chemistry of the soft tissues, and the sites of nucleation of the HAP. By varying the environmental parameters and the biological subjects in such experiments, one could test the importance of many of the environmental and taphonomic requisites that I have suggested were essential to the phosphatization of soft tissues in the Romualdo Member biota. For example, with the use of microelectrodes and other analytical techniques, one could experimentally test the importance of a number of parameters in dictating which organisms, tissues, and biomolecules become phosphatized. In particular, it should be possible to determine the source of phosphorus, the importance and precise role of microbes in inorganic phosphatization, the most favourable chemical microenvironment for mineralization, the extent of chemical compartmentalization in individual carcasses, and the importance of the concentration of phosphorus in the tissues of the organism undergoing phosphatization. Furthermore, by removing samples at various stages of mineralization and examining them in TEM, it should be possible to determine more precisely which matrices

act as nucleation substrates, and follow the development of the various crystal microfabrics that have been identified in the fossil material.

3) The association of microinvertebrates with vertebrates: The microenvironment created around fish carcasses and other vertebrates during the initial stages of putrefaction is critical to the preservation of their soft tissues and the protective growth of early diagenetic concretions. In the Romualdo Member, it would appear that the soft tissues of microinvertebrates (e.g. ostracodes, Bate, 1972; copepods, Cressey and Boxshall, 1989; anostracans, Maisey, 1991, p. 410; and decapod shrimps, Wilby and Martill, 1992) are only preserved when associated with such carcasses. In certain cases, the soft tissues of the invertebrates are preserved in such detail that individual muscle fibres, cell membranes, nuclei, and mitochondria are clearly discernible (Wilby and Martill, 1992). Such associations are relatively common and are therefore potentially valuable sources of data regarding scavenging, parasitism, and predation. Their recovery would also permit more accurate reconstructions of the ecology of ancient communities, and allow the morphology and evolutionary relationships of rarely fossilized taxa to be examined in detail.

Exceptionally well preserved microfossils associated with larger organisms have also been recorded from a number of other deposits. Examples include ostracodes from the stomach region of an ichthyosaur (Dzik, 1978), and giant inoceramids from the Niobrara Chalk Formation (USA) containing commensal fish with fossilized muscle (Stewart, Nat. Hist. Mus., Los Angeles, pers. comm.). Coprolites provide a second, as yet largely unappreciated, mechanism of concentrating microfossils and preserving their soft tissues. A number of biological investigations (see Kornicker and Sohn, 1971) have demonstrated that not only can certain small invertebrates remain in a perfect state of preservation within the alimentary tracts of fish, but that adult ostracodes may be defecated still intact and their eggs remain viable. Ostracodes with phosphatized integument have been reported from the Wealden Beds of Europe (Bertrand, 1903) and are known from spiral coprolites of Triassic age (J.E. Pollard, Manchester University, pers. comm.). Such associations are doubtless widespread in the fossil record, but have not been recorded simply because material is not normally processed in a manner conducive to their recovery.

Examination of fossil material would be complemented by experimental investigations of:

a) The source of phosphorus for the mineralization of the microinvertebrates:

It is likely that the phosphate ions necessary for the mineralization of associated microinvertebrates are provided by the decaying tissues of the "host" organism. This theory could be tested by analysing (colourimetrically) changes in the phosphate flux released from fish carcasses, and the level of apatite supersaturation in the alimentary tract.

b) The rates of decay of microinvertebrates when closely associated with large vertebrates:

The decay rates of microinvertebrates associated with host organisms may be lower than those in the surrounding sediment due to a reduction in the rate of metabolism of the infesting bacteria. This would favour phosphatization by extending the time over which soft tissues are available to be mineralized. Reduced decay rates may result from levels of toxins produced by bacteria metabolizing the soft tissues of the host organism, reaching levels detrimental to the proliferation of microbes infesting the associated organism. The rate of decay of predated organisms in the stomachs of larger organisms may similarly be reduced due to the low pH of the gastric fluids. Rates of soft tissue degradation in small shrimps could be monitored 1) in isolation, and 2) when associated with decaying fish, and compared at the cellular level.

c) Actual attempts at phosphatizing the soft tissues of invertebrates by associating them with vertebrate carcasses:

A series of experiments could explore the controls on the phosphatization of small soft bodied organisms (e.g. worms and arthropods) in association with larger carcasses. These experiments would attempt to reproduce the conditions likely to have led to the preservation of fossil examples (e.g. the Portland Roach and the Romualdo Member). Crustaceans would be introduced into the mantle cavity of decaying bivalves and recovered at specific intervals to be examined for signs of phosphatization on a TEM equipped with facilities for elemental analysis. The effect of a decaying carcass on the decay and mineralization of microinvertebrates would be examined by measuring the Eh, pH, and degree of mineralization of small crustaceans arranged at various distances from a rotting fish. As a control, small crustaceans would also be allowed to decay in the absence of a large carcass.

4) The relationship between the processes of soft tissue phosphatization and other mechanisms of soft tissues mineralization: With the exception of the process of soft tissue pyritization (see Berner, 1980, 1981; Allison, 1988; Briggs *et al.*, 1990), little is known of the mechanisms involved in the replacement of soft tissues by other mineral phases. Certainly there do appear to be close similarities in the preservational styles and fidelity of preservation between soft tissues inorganically phosphatized and those which are silicified (Voigt, 1988). Indeed, Leo and Barghoorn (1976) have experimentally demonstrated the mechanisms involved in the silicification of wood to be remarkably similar to those that I have proposed are active during the inorganic postmortem phosphatization of soft tissues. Leo and Barghoorn (1976, p22, 24) have proposed that reactive head-groups (particularly the hydroxyl group) created during decay in the walls of the plants form hydrogen bonds with silica in solution and thus initiate mineralization. They (Leo and Barghoorn, 1976, p27) also recognised (as do I) the importance of the decay-induced chemical microenvironment and in particular the pH.

A comparative investigation of the preservational styles, replacing microfabrics, and process of silicification would help our understanding of soft tissue fossilization. This study could follow a similar approach to that adopted in this thesis, and could make useful comparisons between silicified plant and animal tissues.

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APPENDIX 1
SPECIMEN DIRECTORY

This appendix lists all of the material figured in volume 2 and referred to directly in the text of volume 1. For each specimen I give: 1) the deposit from which it was collected (unless it is biological material); 2) the collection locality or person/institute from which it was received; 3) a description of its form (i.e. what material is curated); 4) a generic name; 5) its preparation; 6) the specimen number of any accompanying material; and 7) where the specimen is held. I use a number of abbreviations in the tables. These are:

GVB = Glencartholm Volcanic Beds, Scotland G = Gullane shrimp Bed, Scotland

LL = Lower Lias, UK LOC = Lower Oxford Clay, Christian Malford, UK

N = Nova Olinda Member, Crato Formation, NE Brazil P = Portland Roach, UK

R = Romualdo Member, Santana Formation, NE Brazil

S = Solnhofen Limestone, Germany H = Haqel Basin

DNPM = Departamento Nacional da Produçâo Mineral EC = Euan Clarkson

TM = Mr. Terry Manning, Leicester

NO = Nova Olinda, Chapada do Araripe

Pedra Br. = Mina Pedra Branca, Chapada do Araripe

SdoC = Santana do Cariri, Chapada do Araripe

S. Rom. = Sitio Romualdo, Chapada do Araripe

H = hand specimen Z = sectioned S = thin sectioned A = acid residue

Res = residue stubs = SEM stubs M = microtome B999 = resin block

BMNH = British Museum of Natural History OU = Open University

LEIUG = Leicester University

HAND SPECIMENS

Specimen No.	Locality	Description	Preparation	Accomp. Mat.	Curated
PRW/1	R, TM	H, Rhacolepis	Z	S	OU
PRW/2	R, DNPM	H, Notelops			OU
PRW/3	R, Porteiras	H, concretion	Z	S	OU
PRW/4	R, Porteiras	H, Rhacolepis	Z		OU
PRW/5	R, TM	H, Rhacolepis	Z		OU
PRW/6	R, TM	H, Rhacolepis	Z, A	Res, stubs	OU
PRW/7	R, Porteiras	H, Notelops	Z		OU
PRW/8	R, TM	H, Rhacolepis	Z		OU
PRW/9	R, Porteiras	H, Notelops	Z, A	Res, S	OU
PRW/10	R, TM	H, Rhacolepis	Z		OU
PRW/11	R, TM	H, Notelops	Z	M, S, stubs	OU
PRW/12	R, SdoC	H, Vinctifer	Z		OU
PRW/13	R, SdoC	H, Rhacolepis	Z		OU
PRW/14	R, DNPM	H, corpolite	Z, A	Res, S, M	OU
PRW/15	R, DNPM	H, ?Tharrias			OU
PRW/16	R, Rancharia	H, Rhacolepis	Z	S	OU
PRW/17	N, NO	H, Dastilbe	A	Res, stubs	OU
PRW/18	R, Jardim	H, concretion	Z	Res, S, M	OU
PRW/19	R, TM	H, Rhacolepis	Z, A	Res	OU
PRW/20	R, DNPM	H, Cladocyclus	A	Res, stubs	OU
PRW/21	N, NO	H, sediment			OU
PRW/22	R, DNPM	H, Notelops			OU
PRW/23	R, SdoC	H, Lepidotes	Z		OU
PRW/24	R, TM	H, Notelops	A	Res, stubs, M, S	OU
PRW/25	R, DNPM	H, Brachyphyllum	Z	S, stubs	OU
PRW/26	R, Pedra Br.	H, Vinctifer	Z		OU
PRW/27	R, Porteiras	H, Notelops	Z		OU
PRW/28	R, TM	H, Notelops	A	Res, stubs, M	OU
PRW/29	R, TM	H, Rhacolepis	A	Res	OU
PRW/30	R, TM	H, Vinctifer	A	Res, stubs	OU
PRW/31	R, Pedra Br.	H, Notelops	A, Z	M, stubs	OU
PRW/32	R, Porteiras	Notelops	A	Res, M, S	OU
PRW/33	R, Jamacaru	H, Rhacolepis	A, Z, M	Res, M	OU
PRW/34	R, S. Rom.	Rhacolepis	A	Res, stubs	OU
PRW/35	R, Pedra Br.	H, Tharrias	Z, A	Res, stubs	OU
PRW/36	R, TM	H, Rhacolepis	A		OU
PRW/37	R, Porteiras	H, Notelops	A, Z	Res, stubs	OU
PRW/38	R, Porteiras	H, Notelops	A	Res	OU
BMNH P4747	H	S. macrophthalma			BMNH
BMNH P5900	GVB	C. costellatus		stubs	BMNH
BMNH C46871	S	P. prisca		stubs	BMNH
BMNH C46898	LOC	B. antiquus		stubs	BMNH
DM/Lias/2	LL, Dorset	H, undet. ichthyosaur	A	stubs	LEIUG
DM/Santana/15	R, DNPM	H, Vinctifer	Z		OU
DM/Santana/36	R, DNPM	H, Rhinobatos	A		LEIUG
LEIUG107851	R, TM	H, Rhacolepis	A	Res, stubs, M	LEIUG
LEIUG110562	R, Porteiras	H, Rhacolepis			LEIUG
CPCR801	R, DNPM	H, Vinctifer			DNPM
CPCR2413	R, DNPM	H, Tharrias			DNPM
CPCR88	R, DNPM	H, Tharrias			DNPM
DNPMD6M488LE	R, DNPM	H, undet. pterosaur		DNPMD6M488LE	DNPM

SEM STUBS

Stub Number	Local.	Description	Prep.	Accomp. Mat.	Curated
2	R	undet. shrimp chelicera	A	LEIUG107851	OU
3	R	undet. shrimp exuvae	A	LEIUG107851	OU
7	R	undet. shrimp	A	LEIUG107851	OU
9	R	Brachyphyllum		PRW/25	OU
10	R	Vinctifer skin	A	PRW/30	OU
12	R	undet. shrimp	A	LEIUG107851	OU
12	R	undet. shrimp	A	LEIUG107851	OU
13	GVB	Ctenacanthus costellatus muscle	A	BMNH P5900	OU
14	R	undet. shrimp	A	LEIUG107851	OU
18	R	undet. shrimp eggs	A	PRW/37	OU
20	R	Rhacolepis hypodermis	A	LEIUG107851	OU
26	R	Notelops skin	A	PRW/28	OU
27	R	Rhacolepis skin	A	LEIUG107851	OU
28	R	Rhacolepis gut	A	PRW/6	OU
38	R	Rhacolepis muscle	A	PRW/34	OU
51	R	Rhacolepis muscle	A	LEIUG107851	OU
53	R	Notelops muscle	A	PRW/24	OU
54	R	Notelops muscle	A	PRW/31	OU
64	R	Tharrias gills	A	PRW/35	OU
66	R	gastric residue	A	PRW/35	OU
78	R	Cladocyclus muscle	A	PRW/20	OU
79	R	Rhacolepis muscle	A	LEIUG107851	OU
79	R	Rhacolepis muscle	A	LEIUG107851	OU
81	R	Notelops muscle	A	PRW/24	OU
86	R	Notelops gills	A	PRW/11	OU
89	LOC	Belemnotheutis antiquus muscle	A	BMNH C46898	OU
91	S	Plesioteuthis prisca muscle	A	BMNH C46871	OU
96	R	Notelops muscle	A	PRW/28	OU
101	R	Notelops gut	A	PRW/31	OU
102	R	Notelops artery	A	PRW/31	OU
103	R	Rhacolepis muscle	A	PRW/6	OU
112	LL	undet. ichthyosaur soft tissues	A	DM/Lias/2	OU
113	LL	undet. ichthyosaur soft tissues	A	DM/Lias/2	OU
116	P	L. gibbosa gut	A		OU
117	P	L. gibbosa mantle	A		OU
118	P	L. gibbosa mantle	A		OU
120	N	Dastilbe muscle	A	PRW/17	OU
126	R	Cladocyclus muscle	A	PRW/20	OU
133	R	undet. bivalve	A	PRW/6	OU
135	R	Notelops gut	A	PRW/31	OU
137	G	Telliocaris			OU
142	R	Notelops gut	A	PRW/31	OU
145	R	Notelops muscle	A	PRW/28	OU
148	P	L. gibbosa gut	A	Res148	OU
158	P	ooliths	A	Res158	OU
212	R	Cladocyclus blood vessel	A	PRW/20	OU
223	GVB	Ctenacanthus costellatus muscle	A	BMNH P5900	OU
LEIUG107847	R	Rhacolepis stomach	A	LEIUG107851	LEIUG
LEIUG107848	R	undet. shrimp eye	A	LEIUG107851	LEIUG
LEIUG107849	R	undet. shrimp musclature	A	LEIUG107851	LEIUG
LEIUG107850	R	undet. shrimp	A	LEIUG107851	LEIUG
DNPMD6M88LE	R	undet. pterosaur	A	DNPMD6M88LE	DNPM

MICROTOME SECTIONS

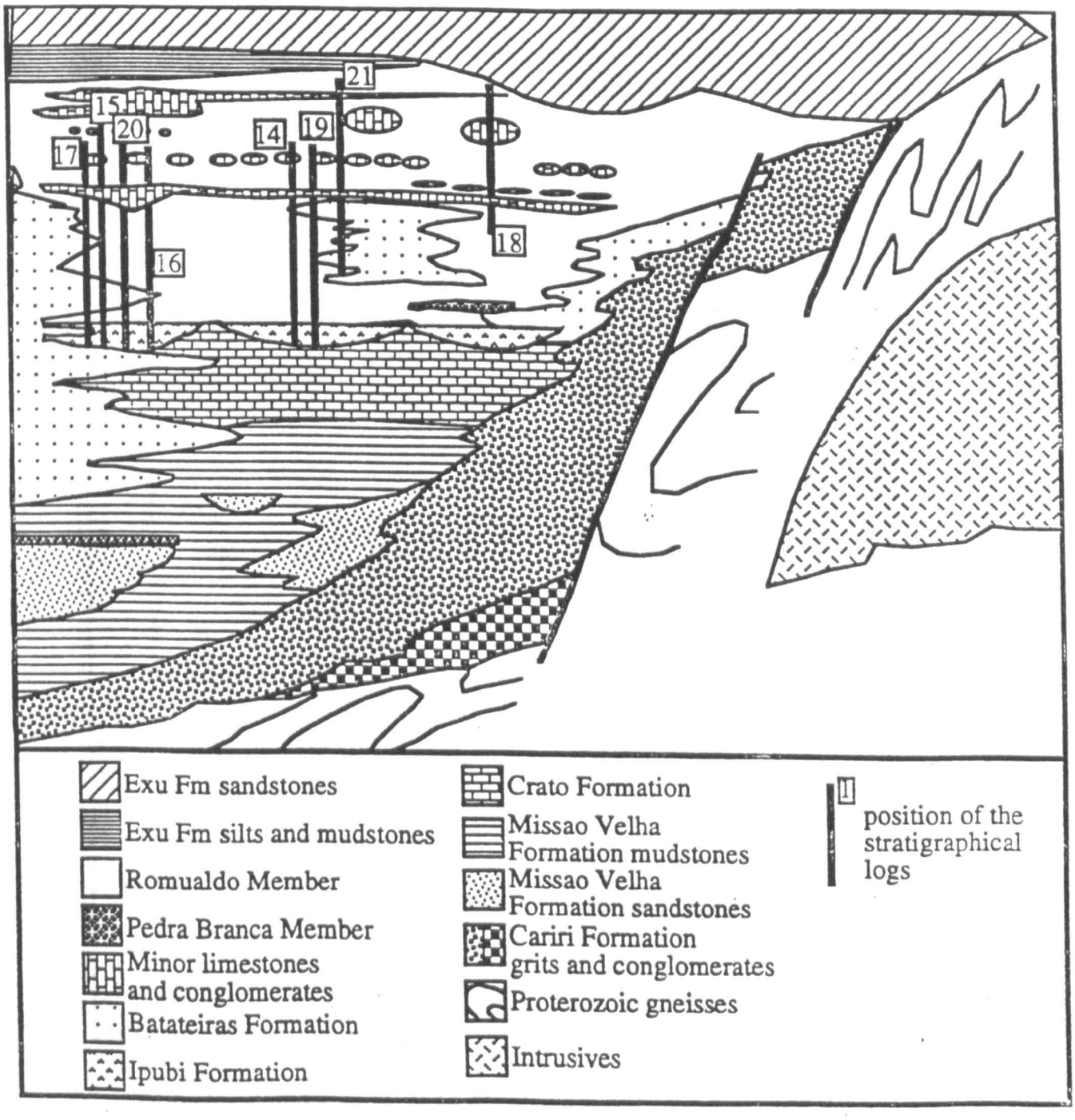
Number	Local.	Description	Preparation	Accomp. Mat.	Curated
A1	M	Rec., Gadus sp. muscle	M	B582	OU
A3	M	Rec., Gadus sp. muscle	M		OU
A5	M	Rec., Gadus sp. muscle	M	B574	OU
A7	M	Rec., Gadus sp. muscle	M	B620	OU
A9	M	Rec., Gadus sp. muscle	M		OU
B2	M	Rec., Gadus sp. muscle	M	B579	OU
B4	M	Rec., Gadus sp. muscle	M		OU
B6	M	Rec., Gadus sp. muscle	M		OU
B8	M	Rec., Gadus sp. muscle	M		OU
B10	M	Rec., Gadus sp. muscle	M		OU
C1	M	Rec., Gadus sp. muscle	M	B604	OU
C3	M	R, Notelops muscle	A, M	B479, PRW/24	OU
C5	M	R, Notelops gills	A, M	PRW/28	OU
C7	M	R, fish muscle	A, M	PRW/18	OU
C9	M	R, fish muscle	A, M	PRW/18	OU
D2	M	R, coprolite	A, M	PRW/18	OU
D4	M	R, coprolite	A, M	PRW/14	OU
D6	M	R, Notelops muscle	A, M	PRW/32	OU
D8	M	R, Notelops muscle	A, M	PRW/11	OU
D10	M	R, Rhacolepis muscle	A, M	PRW/33	OU
E1	M	R, Notelops muscle	A, M	PRW/28	OU
E3	M	R, Notelops sarcolema	A, M	PRW/32	OU
E5	M	R, Notelops skin	A, M	PRW/32	OU
E7	M	R, Notelops muscle	A, M	B560, PRW/24	OU
E9	M	R, Notelops muscle	A, M	PRW/24	OU
F2	M	R, Notelops gut	A, M	B480, PRW/31	OU
F4	M	R, Rhacolepis muscle	A, M	LEIUG107851	OU

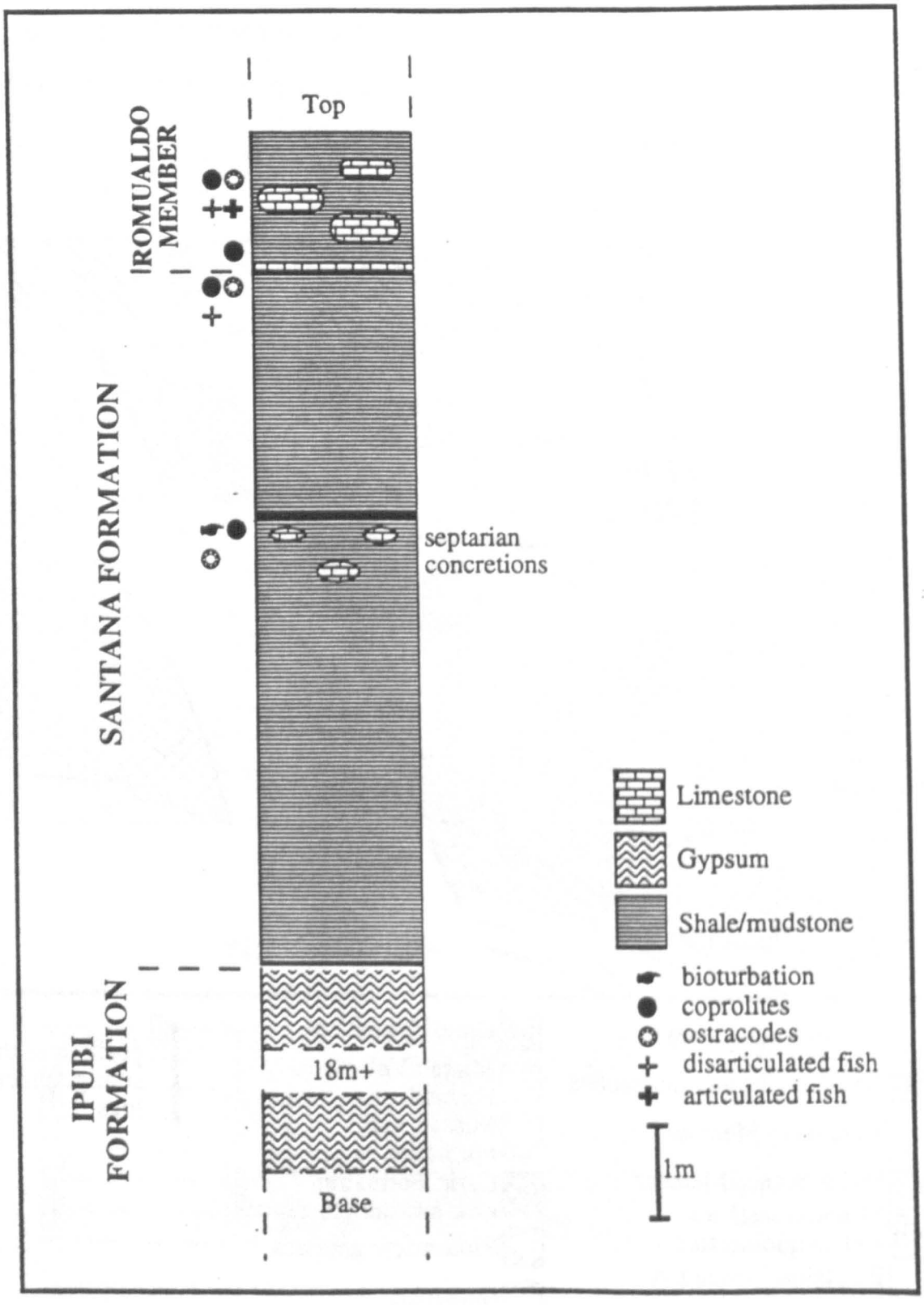
PETROGRAPHICAL SLIDES

Slide Number	Locality	Description	Accomp. Mat.	Curated
A	R, DNPM	coprolite	PRW/14	OU
B	R, Porteiras	Notelops		OU
C	R, TM	Notelops	PRW/11	OU
D	R, Porteiras	Notelops	PRW/32	OU
E	R, TM	Notelops	PRW/11	OU
F	R, Porteiras	Notelops	PRW/32	OU

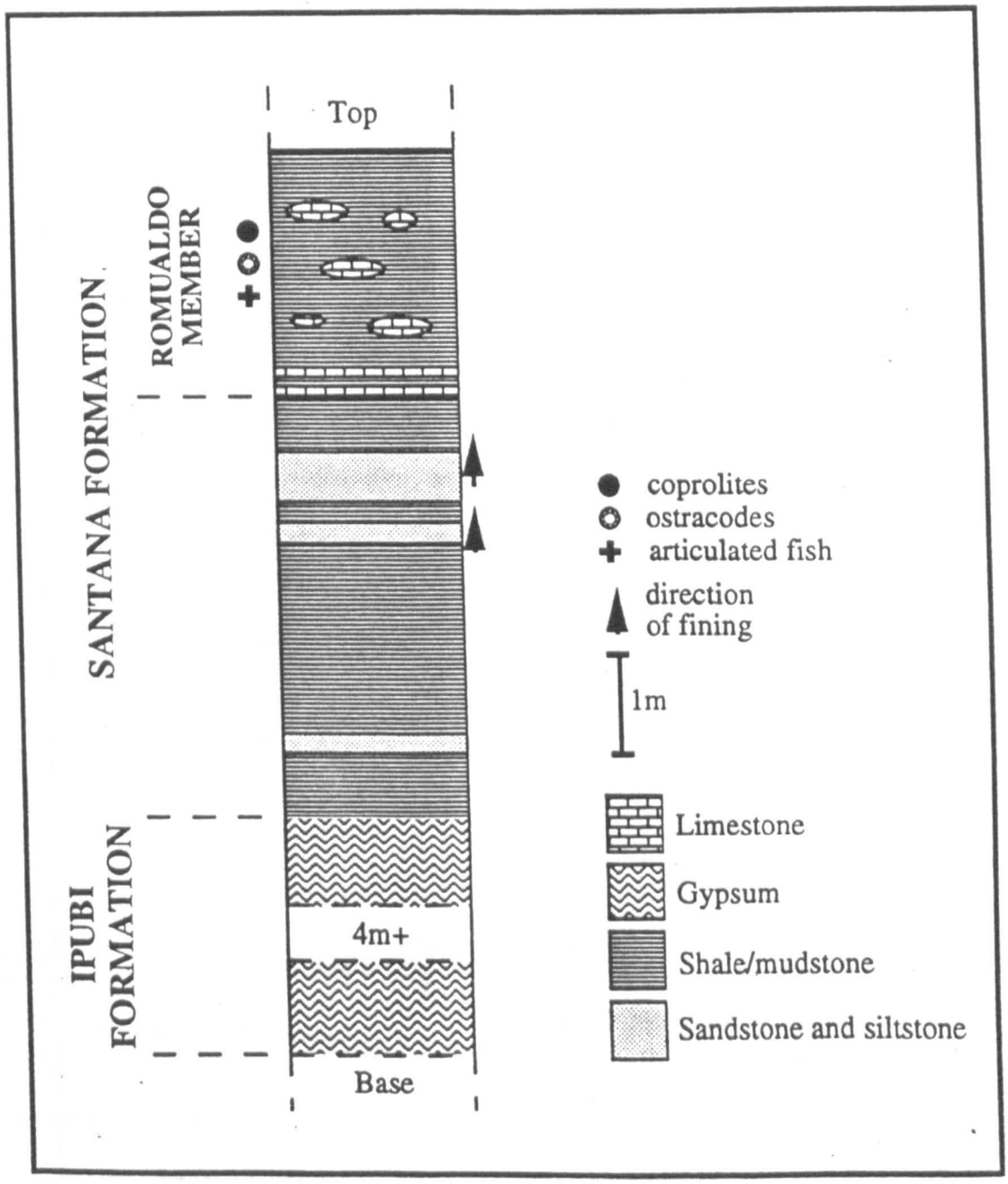
APPENDIX 2
STRATIGRAPHICAL LOGS

All of the stratigraphical logs not referred to directly in the text (but none the less were important in reviewing the Araripe Basin's stratigraphy) are given in this appendix. The locality of each log is given in text figure 2.1, and their positions in the sedimentary sequence is given in text figure App.2.i.

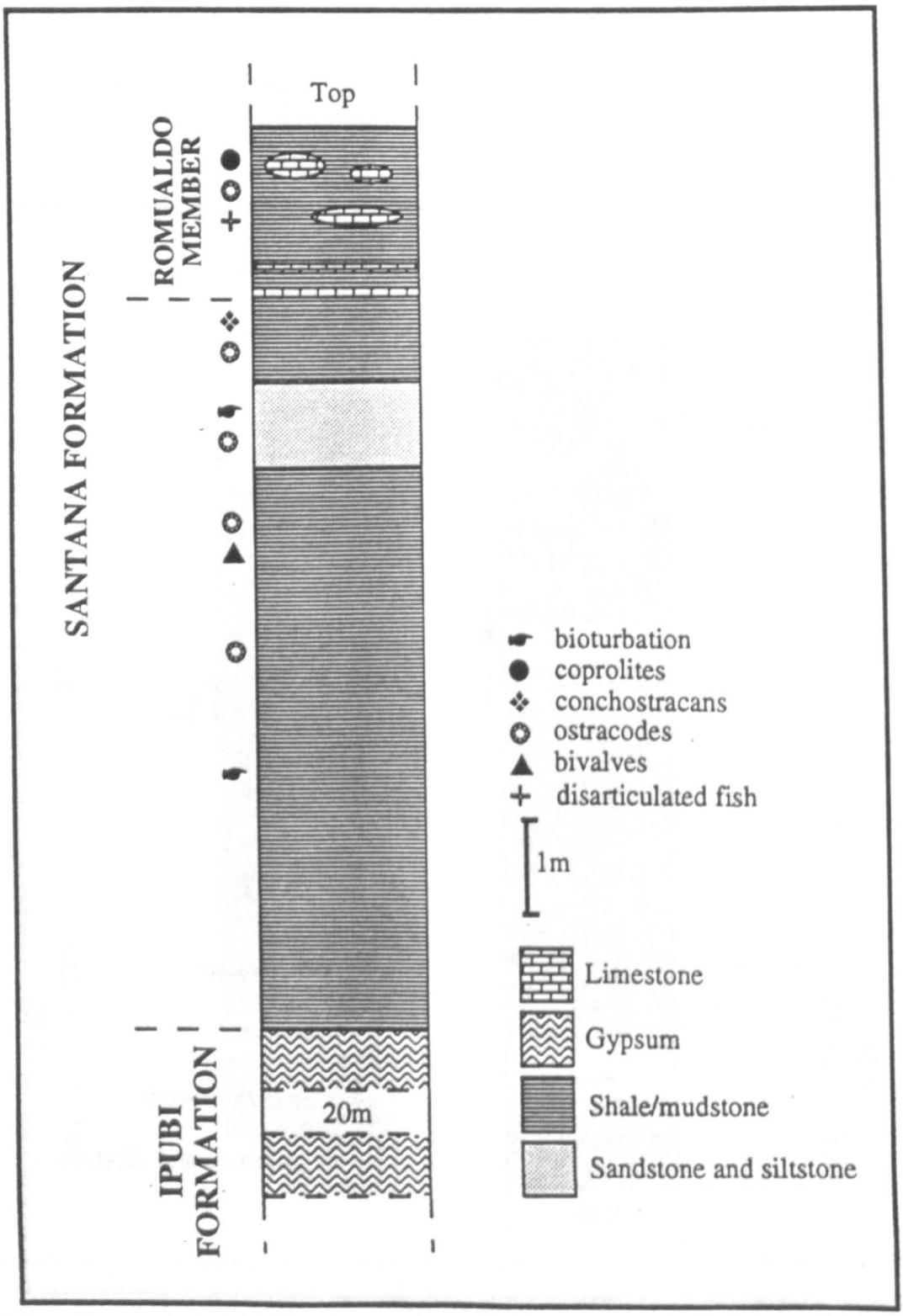




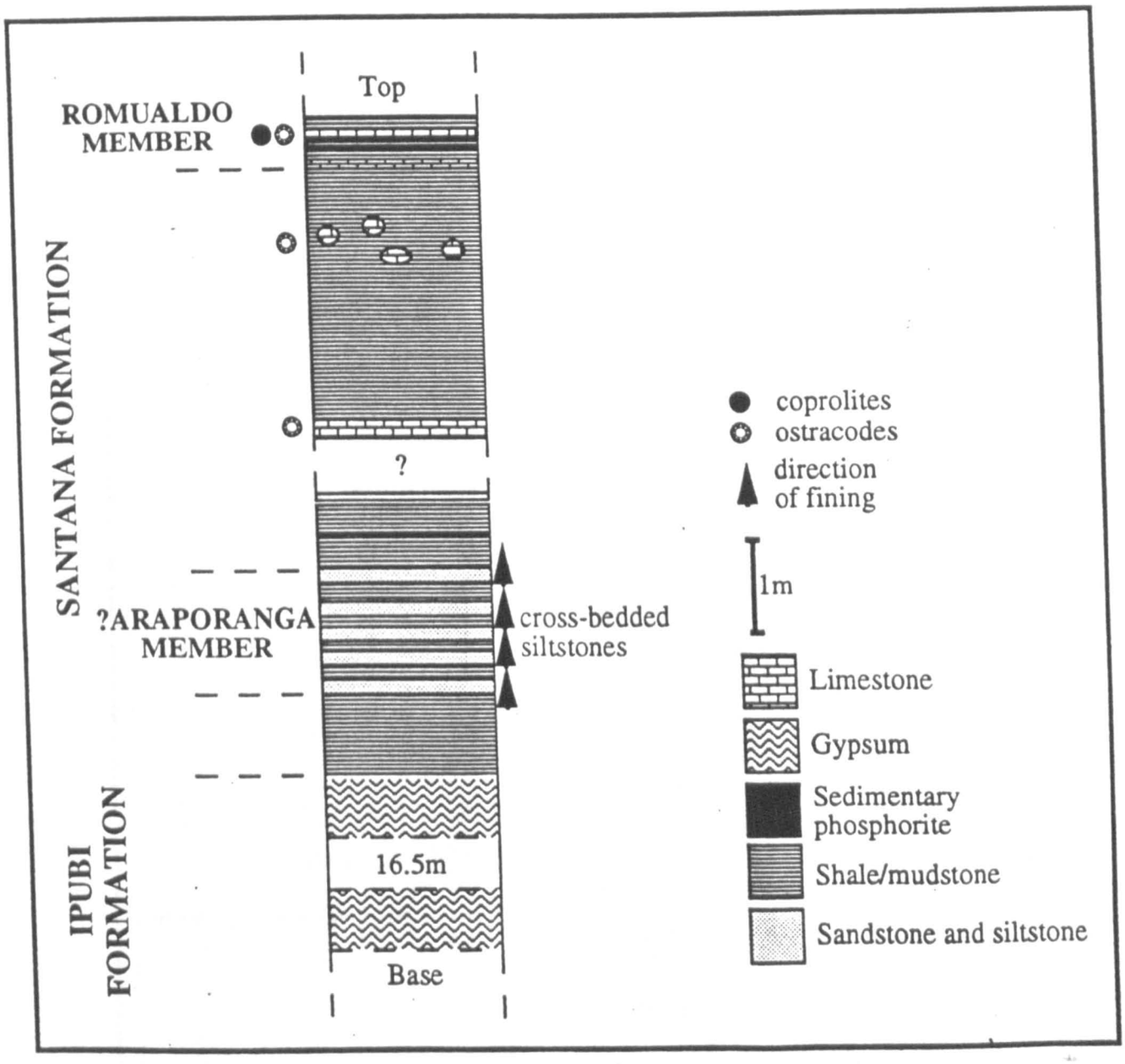
Text figure App.2.ii: Sedimentological log of locality 14, a new quarry midway between Mina Alto Bonito and Mina Severino, Ipubi.



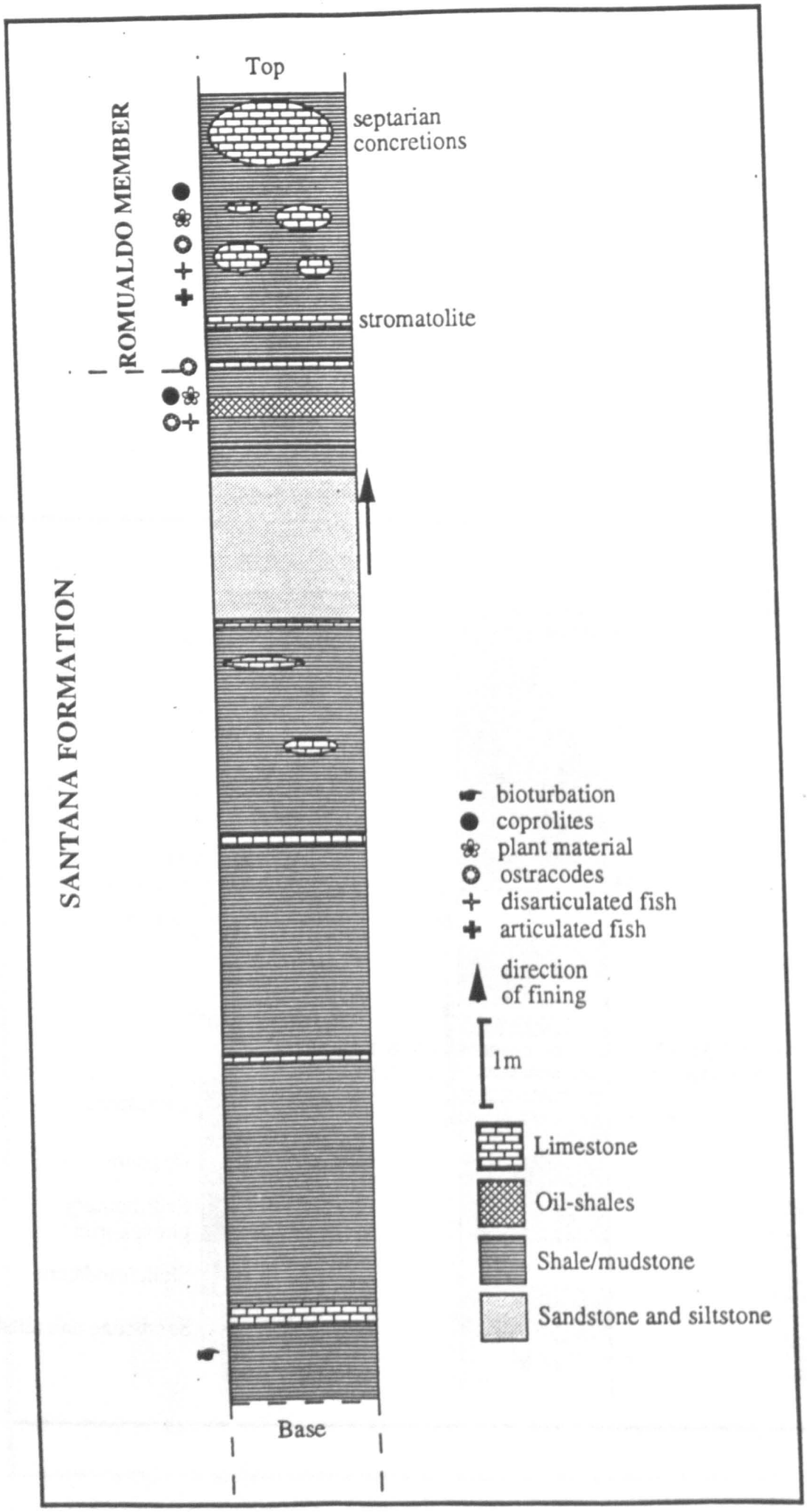
Text figure App.2.iii: Sedimentological log of locality 15, Mina do Gapim, Araripina.



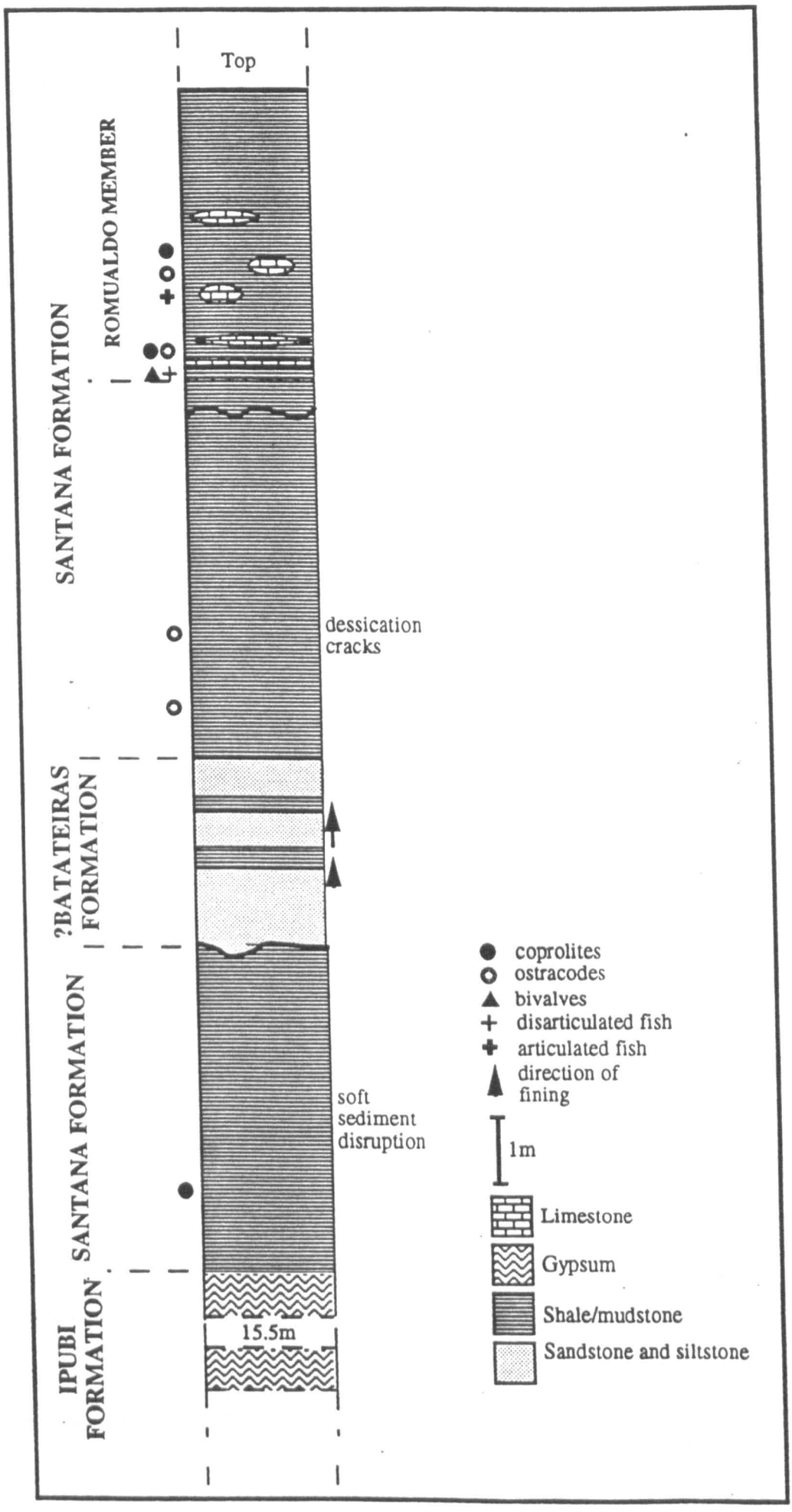
Text figure App.2.iv: Sedimentological log of locality 16, Mina Rancharia, Rancharia.



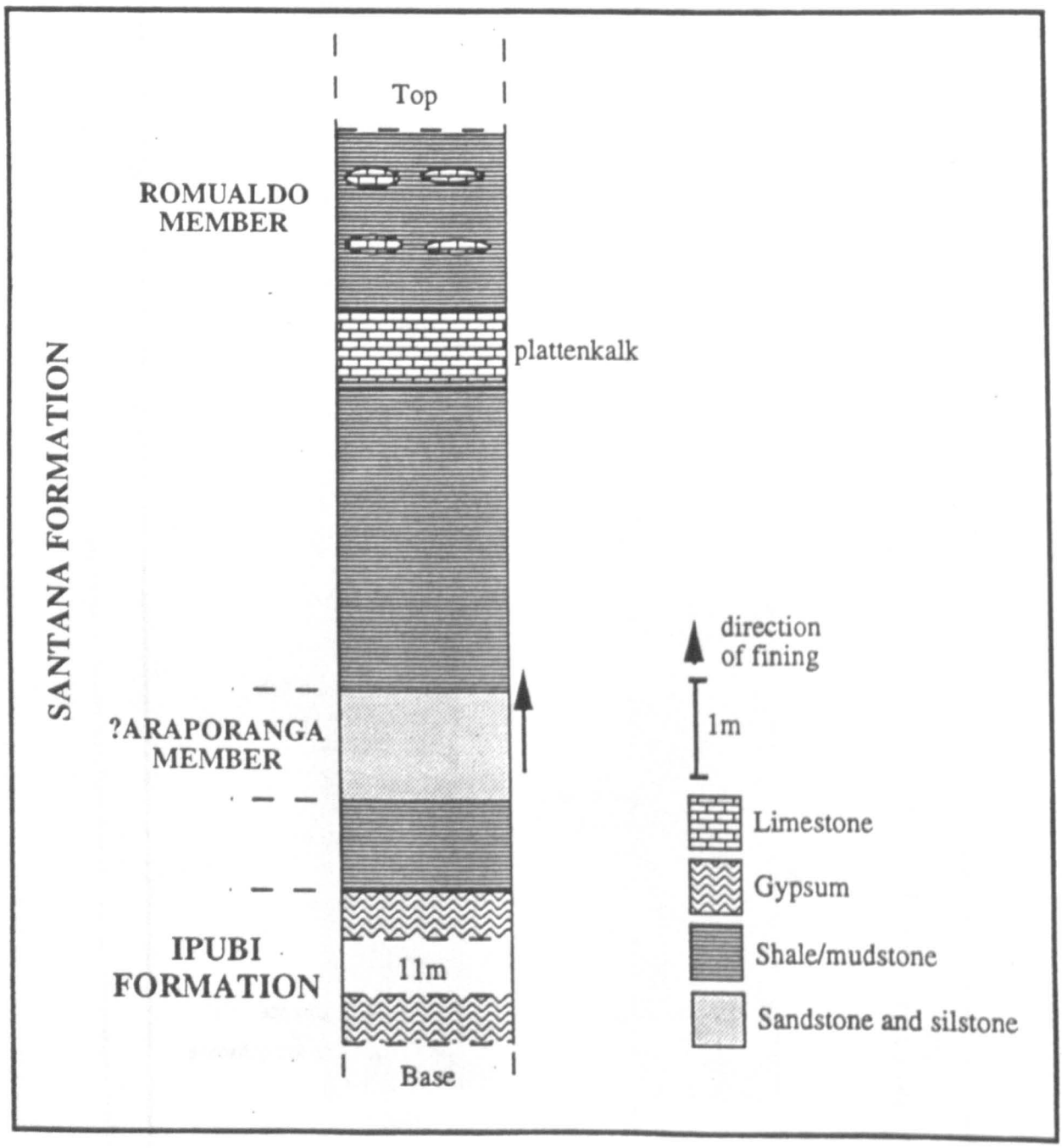
Text figure App.2.v: Sedimentological log of locality 17, Mina Case de Pedra, Ipubi.



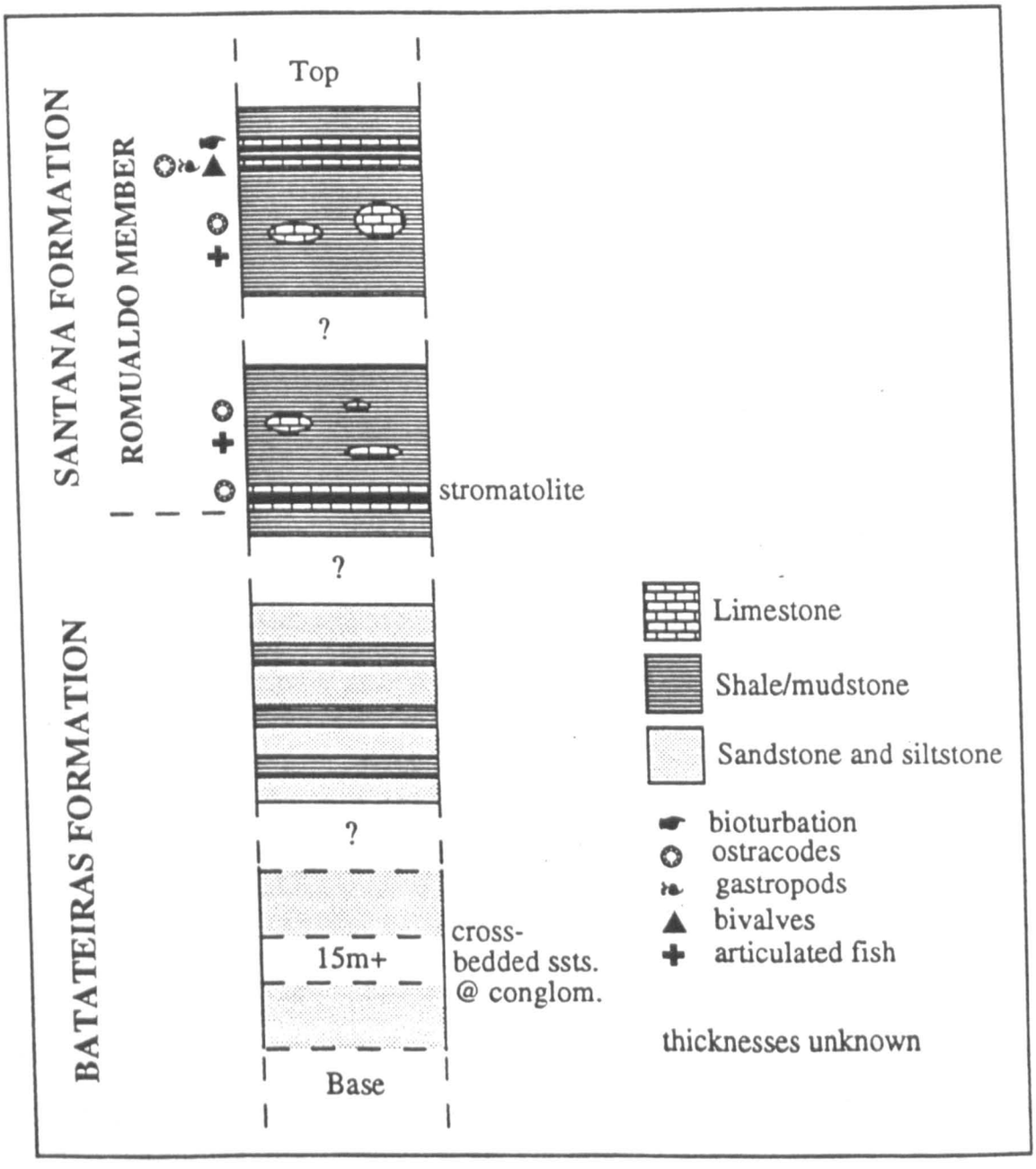
Text figure App.2.vi: Sedimentological log of locality 18, a stream section at Jamacaru.



Text figure App.2.vii: Sedimentological log of locality 19, Mina Sao Jorge, Ipubi.



Text figure App.2.viii: Sedimentological log of locality 20, Mina do Jasias, Rancharia.



Text figure App.2.ix: Sedimentological log of locality 21, an old quarry at Sitio Romualdo.

APPENDIX 3
SAMPLE PREPARATION

i) ENERGY-DISPERSIVE ANALYSIS of X-RAYS (EDAX)

This technique permits the mapping of a number of elements at any one time from carbon-coated petrographic thin sections. With respect to fossil specimens, it is of particularly interest since it allows the examination of material *in situ* and therefore in its original relation to diagenetic minerals and sedimentary structures. However, with the exception of a few authors (see Aldridge and Armstrong, 1981), the technique has as yet received little attention from palaeontologists although its application to the examination of phosphatized soft tissues which are too delicate to withstand acid digestion (see Müller, 1985) has recently been described by Martill *et al.* (1992).

The potential of this technique for studying exceptional fossil material need not necessarily be restricted to phosphatized soft tissues; it may be of equal use in the examination of lightly silicified or pyritized material. The basic requirement is merely a difference in the mineralogy between the fossil material and the host sediment.

Müller (1985) has suggested that deposits containing phosphatized arthropods may be considerably more abundant than previously envisaged, and are simply not being identified due to the faunas' small size and excessive delicacy. It is anticipated that EDAX elemental mapping may also be helpful in assessing more accurately the true stratigraphical distribution of such material.

The Physics of the Process: The technique of microanalysis relies on electrons of the appropriate energy, when impinging upon a sample, causing the emission of X-rays whose energies and abundance are dependent on the composition of the sample. The specifics are discussed in more detail elsewhere (Anon., 1983 and Goldstein *et al.*, 1981)

Method: Uncovered glass-mounted petrographic thin sections of around 50μm thickness were carbon coated (approximately 2 mins.) and placed in a large specimen holder around which aluminium foil was packed to increase electrical conductivity. Analyses were performed in a JEOL 820K SEM equipped with a Kevex energy dispersive microanalytical system composed of a Kevex Super Quantum Detector and a Kevex Delta 4 Processor. Thin

sections of standard thickness (30μm) were abandoned in favour of the thicker sections in an attempt to minimise adhesive interference. This was particularly prevalent at accelerating voltages in excess of 20Kv where the electron/specimen interaction volumes penetrated to a depth in excess of 30μm (Goldstein *et al.*, 1981) and excited the adhesive's constituent elements.

Optimum accelerating voltages are therefore a rather delicate interplay between exciting the required elements sufficiently to fluoresce (generally 2.5 times their peak values) and not producing surface charging, specimen damage, or exciting too large an area. Sampling times were kept to a minimum (1.5 μsecs) in order to reduce mapping times. This does not significantly affect the resolution of the final image. In general, accelerating voltages of 15-20Kv are required to excite Ca and P. Maps for these elements were acquired over periods ranging from 2 mins. (reconnaissance maps) and 2 hrs., but were generally adequate after 10 mins.

An advantage with the Kevex system is that up to sixteen elements may be simultaneously mapped and displayed on a split screen, thereby permitting direct comparisons of their relative distributions. Areas of greatest abundance of the mapped element are distinguished from those of reduced abundance by differences in the brightness (spot density) of the image. Increased abundance is signalled by an increase in brightness. The validity of this was established by ZAF (Atomic number Absorbtion Fluoresence) correction and by the examination of the same area at a number of different Kv's and orientations. Slight variations in the surface topography of the thin section (produced by the preferential 'plucking-out' of phosphatized soft tissues during grinding) and the continuation of tissues below the surface of the thin section (beneath other features) appears not to produce undue interference. The most favourable results were obtained at magnifications between 20 and 200 times. At higher magnifications, resolution is considerably reduced.

Despite its enormous potential, this technique has one draw-back; it is extremely time consuming, particularly when trying to identify potentially informative areas with reconnaissance maps. This problem, to some extent, can be diminished by sketching the sections and marking key areas with felt-tip pens prior to SEM examination. Back-Scattered images can also provide some clues as to profitable sites for mapping and are therefore

frequently recorded with elemental maps. However, due to the low density contrast between calcite and calcite/apatite intergrowths, they are considerably less informative than the elemental maps themselves.

ii) CRITICAL POINT DRYING (CPD)

Critical Point Drying offers a relatively quick and simple method of preparing recent biological soft tissues (saturated with water) for scanning electron microscopy. It offers the palaeobiologist a means of looking at comparative biological material in the same form and by the same means as fossil specimens. It is therefore ideal for comparative taphonomic and anatomical studies, but with the exception of Hill (1987) and Martill and Harper (1990), it has received little attention from palaeontologists.

Physics and Method: CPD involves elevating the liquid in which the specimen is saturated to its Critical Point. All fluids have a Critical Temperature (T_c) and Critical Pressure (P_c) such that the surface tension is zero and the liquid turns imperceptibly into a gas when both are reached (the Critical Point). Thus the liquid is removed from the tissue without the gross damage caused by the surface tension associated with air drying. Water is an impractical ambient fluid (T_c=374^{0}C, P_c= 217.7 Atm), therefore the lower T_c (31^{0}C) and P_c (72.9 Atm) of carbon dioxide is exploited.

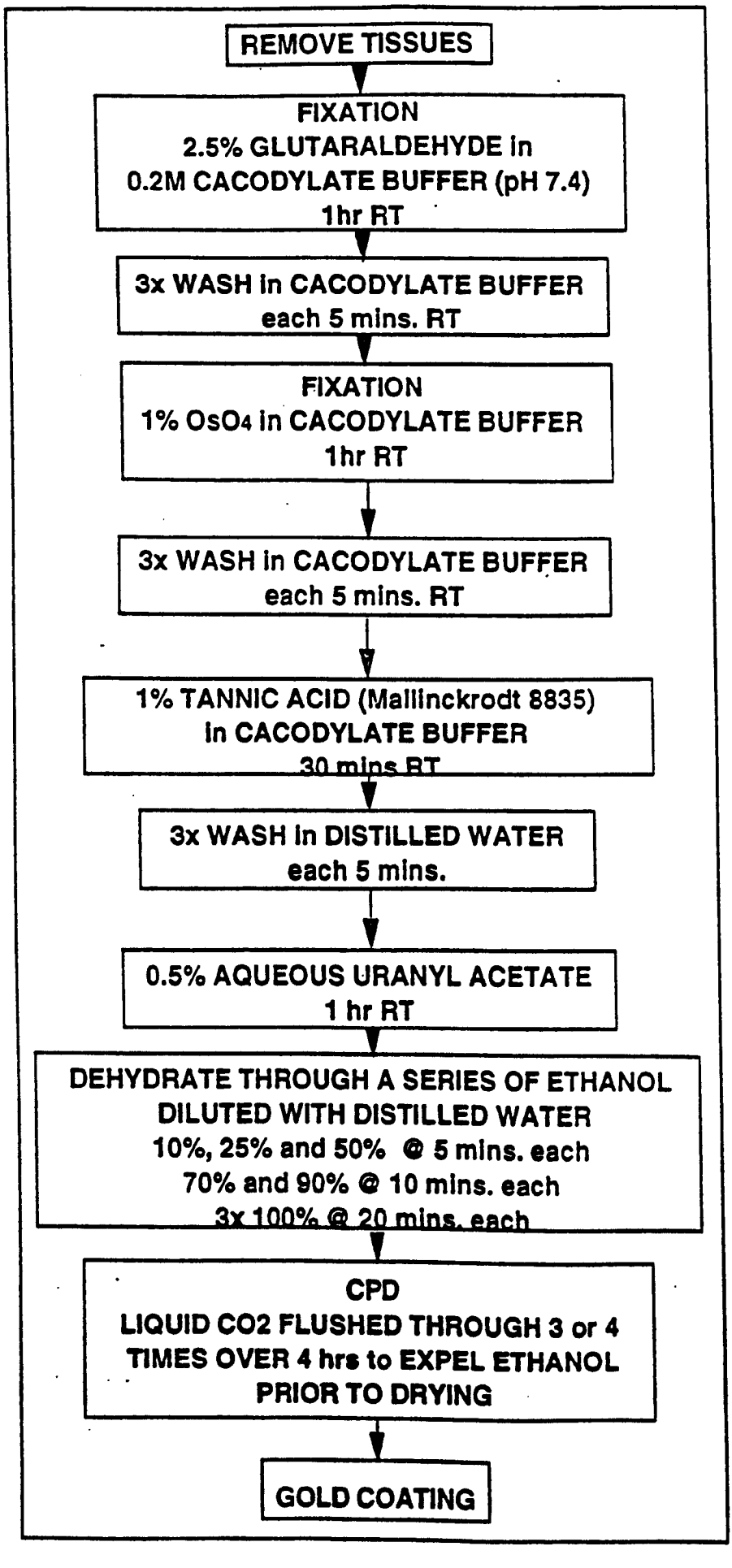
The specifics of preparation (based on Wollweber *et al.*, 1981) are given in text fig. App. 3ii.

Notes:

1) It is important that pure (analytical grade) ethanol is used in at least the final stage of dehydration so that deposition of contaminants on the surface of the tissue is prevented.

2) The thorough washing of specimens after 'osmication' is essential to prevent of the deposition of residual osmium as salts on the tissues.

3) Considerable care must be exercised throughout the procedure to minimize the production of artefacts. In particular, specimens must be relatively small (<5mm^3), be extremely permeable (e.g. branchial apparatus), or have a large surface area in relation to volume to enable sufficient exchange of reagents. At no time must the specimens be allowed to remain out of solution for extended periods.



Text figure App. 3ii. Schedule for the CPD of biological tissues

4) Some observational problems may develop with progressively more degraded tissues in taphonomic experiments. Frequently, 'bacterial scums' and decay products may obscure surface features. This to some extent may be minimized by washing the specimen in the ambient fluid prior to fixation. Additional extraneous material may also be removed after drying by *gently* touching the surface with masking tape.

iii) RESIN EMBEDDING:

Examination of tissues at high magnifications on TEMs requires their fixation and embedding in resin. This is a standard biological technique and involves the production of extremely thin (<70nm) sections. Different procedures are presented here to enable the examination of both 'fresh' and fossil soft tissues.

a) Notes on the preparation of Recent tissues: Glauert (1974) gives a general account of the processes of fixation, dehydration, and embedding of biological specimens; and Hayat (1970) describes various staining procedures. The processing schedule given in text fig. App. 3iiia is a slightly modified version of those given in the two forenamed references.

1) Sections were examined at 60Kv.

2) Difficulties associated with the thorough fixation of extensively decomposed tissues were experienced. These can be minimized by processing extremely small ($\approx 1mm^3$) pieces of material and by periodically replacing the fixatives with fresh solutions during fixation.

b) Notes regarding the processing of fossil material: protocol given in text fig. App. 3iiib.

1) Due to the extremely friable nature of the fossil material under examination, extreme care must be exercised at all times, particularly when changing resins. Mixing rotators must be maintained at their slowest possible speeds.

2) A number of different resins were experimented with; TAAB Transmit EM Resin[1] gave the best results due to its extreme hardness. This reduces the preferential 'plucking-out' of the fossil material.

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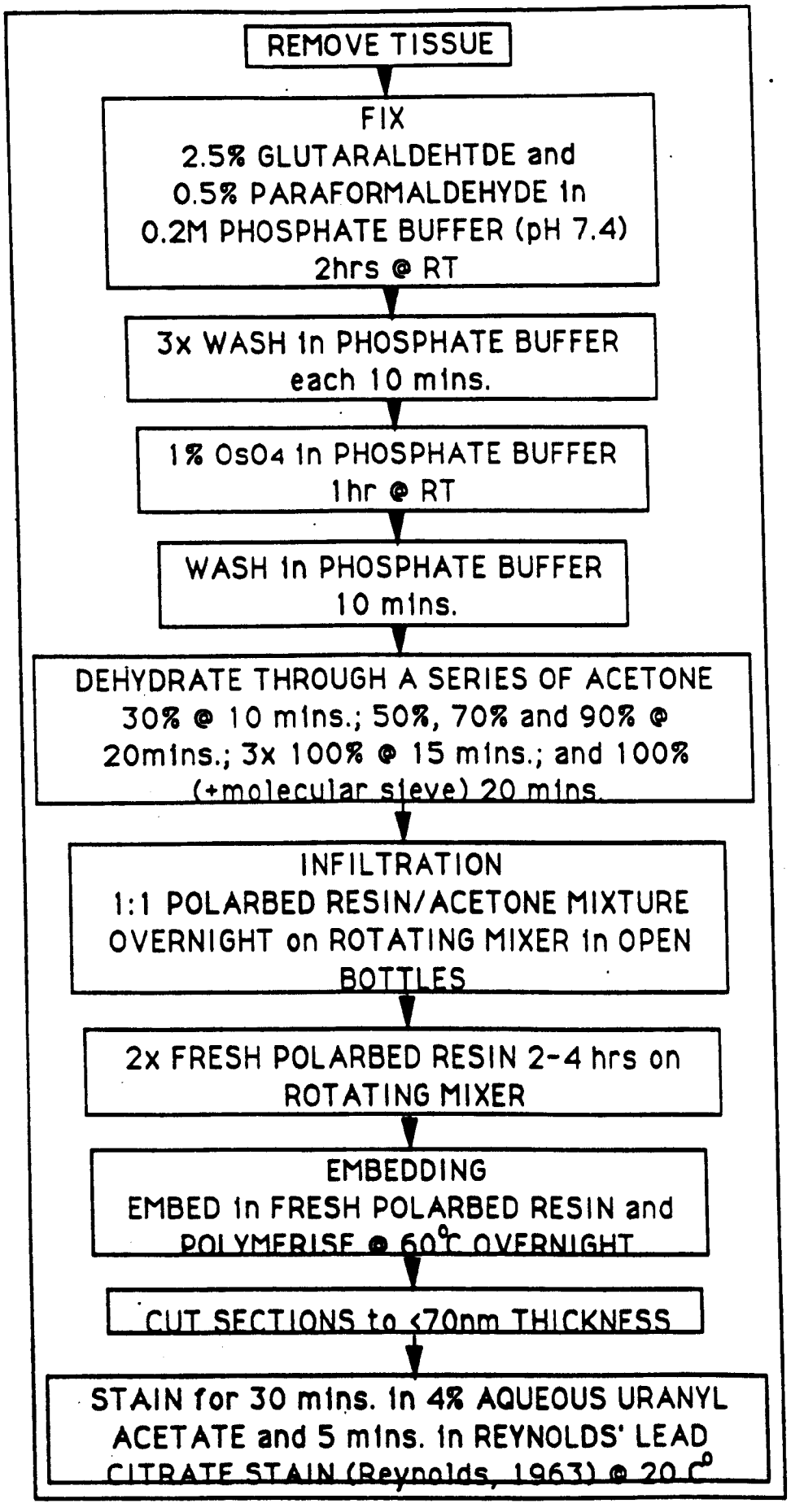
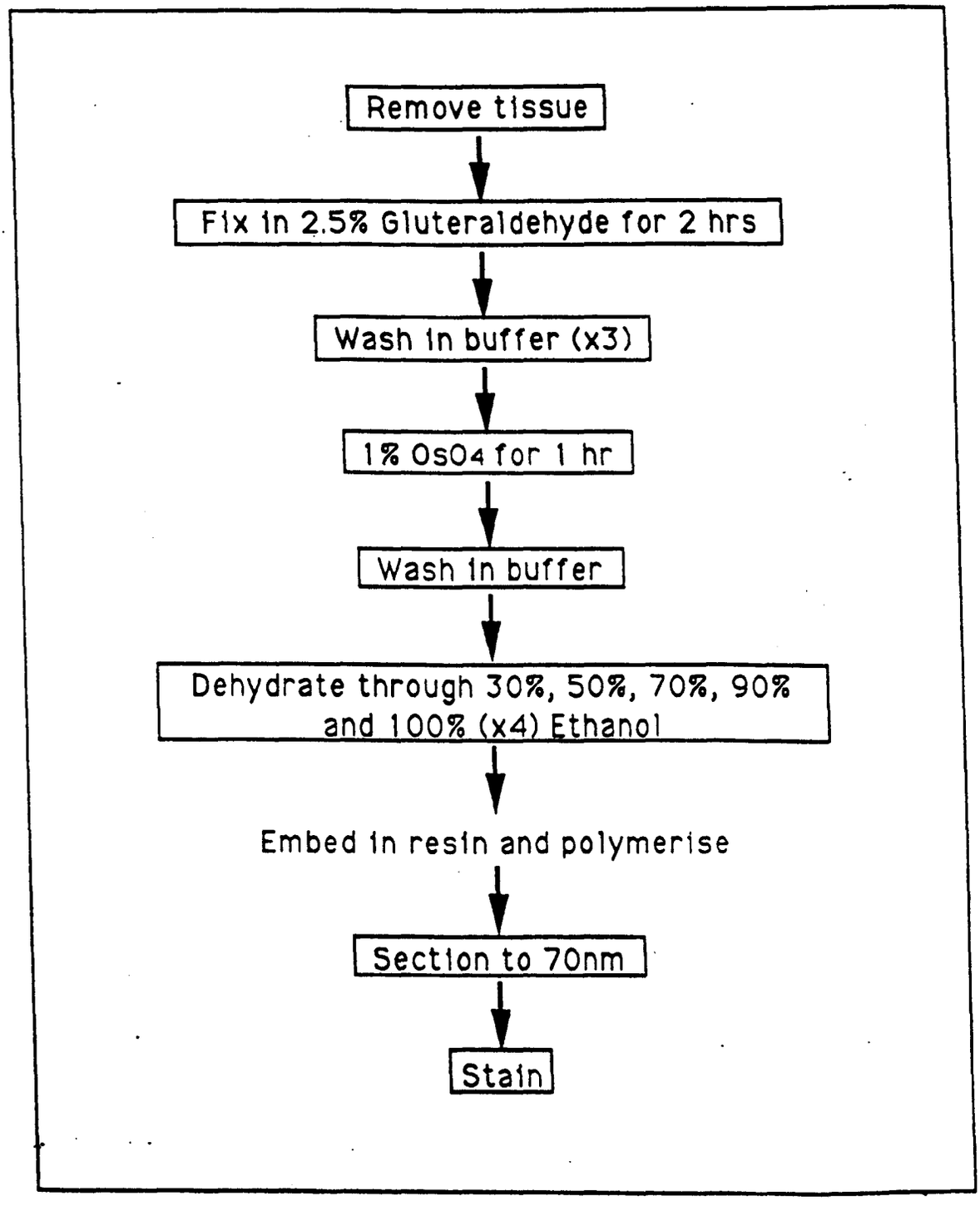
[1]TAAB Laboratories Equipment Ltd, 40 Grovelands Rd, Reading, Berkshire.



Text figure App. 3iiia. Processing schedule for resin embedding

Recent tissues



Text figure App. 3iiib. Processing schedule for resin embedding fossil phosphatized soft tissues

3) Gravity-fed microtomes are recommended over motorized models since the downstroke of the latter is considerably weaker than the former and is therefore likely to 'struggle' when sectioning apatitic material.

4) Material for examination should be kept as small as possible to reduce the production of holes during sectioning. Although high accelerating voltages (200Kv) are necessary to examine this material, support films were not used since these markedly reduce the resolution obtained. Unfortunately, this makes the sections particularly prone to beam damage. It is therefore recommended that they are not left unattended in the column for extended periods.

5) Despite rapid wear, glass knives are recommended for phosphatic material rather than costly, and delicate diamond blades.

The mechanisms and timing of mineralization of fossil phosphatized soft tissues

A thesis presented for the degree of

Doctor of Philosophy

by

PHILIP RICHARD WILBY

B.Sc. (Hons.) Leics. 1989

Department of Earth Sciences

The Open University

August 1993

Volume 2 of 2

FIGURES VOLUME

List of Figures

KEY TO THE FIGURES

Due to the inevitable loss of resolution which accompanies the production of plates and photocopies, all figures in this thesis are deliberately given as original prints. Although this makes for a rather cumbersome volume, it ensures that the important features of each figure are illustrated with the greatest clarity. Unfortunately, the contrast of many of the photomicrographs given is not as great as would have been liked. This is the result of a technical fault in the SEM.

Unless otherwise indicated, all macro-specimens figured in this volume are the correct way up. All figures either contain a scale, or one is given in the accompanying caption. With the exception of the scale bar and the Kv reading, all other data given in the EM photomicrographs should be ignored. Each figure is accompanied in the caption by a specimen number. These are tabulated in Appendix 1.

All of the EDAX elemental maps given consist of two or three different elemental maps of the same area of a carbon coated thin section. The areas of greatest abundance of each element are those of greatest brightness (or highest spot density)(see Appendix 3i).

I have used a number of abbreviations in the figure captions to describe the format of each sample and the technique by which they were examined. These are:

SEM = an SEM photomicrograph

TEM = a TEM photomicrograph

Slide = a petrographical slide

stub = an SEM stub

Micro = an ultrathin section cut on a microtome and mounted on a grid

Biological = Recent material sampled from taphonomic experiments

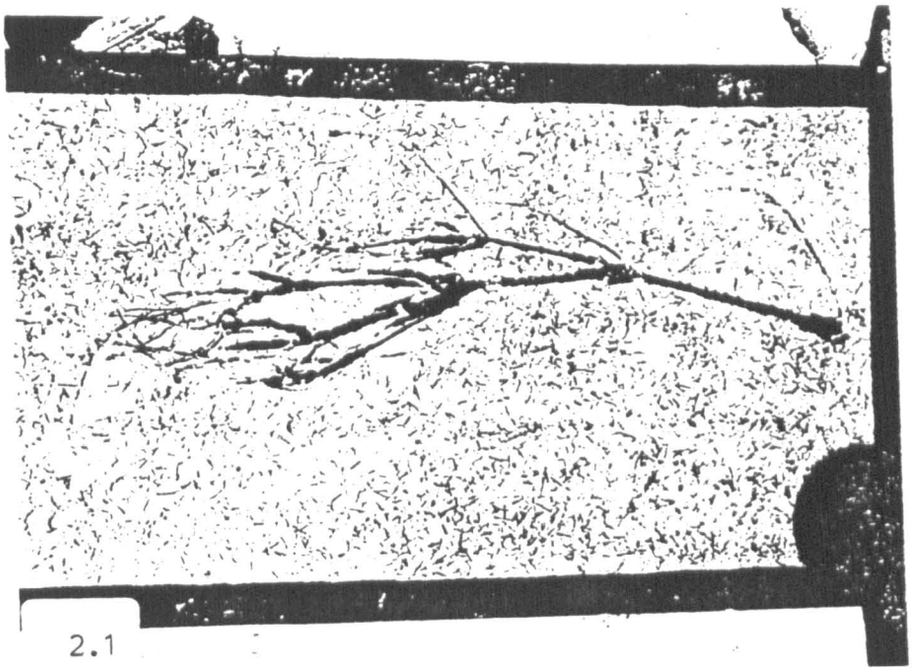
Destroyed = the specimen was destroyed in the course of its examination

Reference to the relevant text in Volume 1 should be sought for a more detailed discussion of the figures.

Figure 2.1: A creased and teared microbial mat in the plattenkalks of the Nova Olinda Member, Crato Formation. The microbial mat is prescrved as an impression on the underlying carbonate lamina. PRW/21

Figure 2.2: Exceptionally well preserved dragonfly (undet. spec.) from the Nova Olinda Member, Crato Formation. Specimen held in the Santana do Cariri Museum: no identification number.

Figure 2.3: Exceptionally well preserved branching stem of an ?angiosperm from the Nova Olinda Member, Crato Formation. The bedding plane is covered in small (≈2x7mm), simple, stick-like ?algal filaments. Specimen held in the Santana do Cariri Museum: no identification number. Lens cap for scale.



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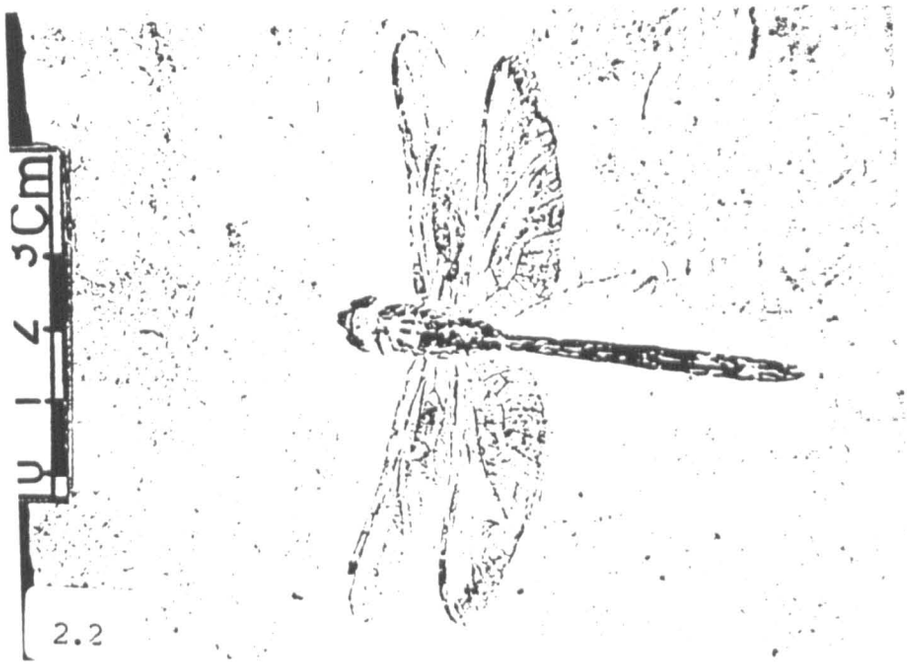

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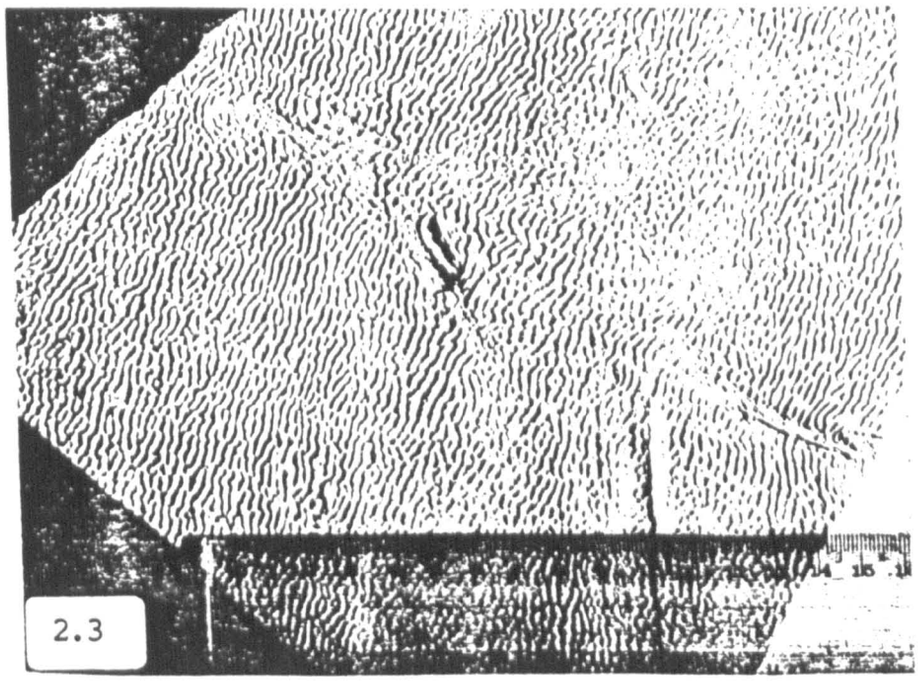


2.3

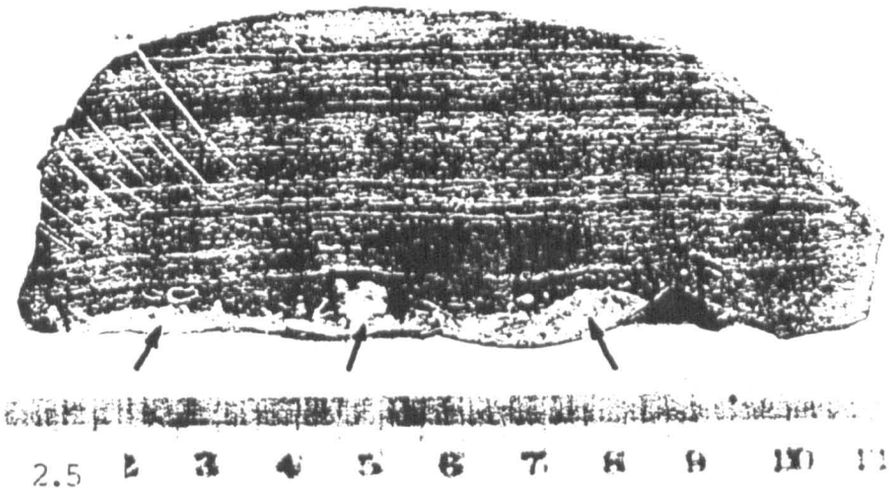
Figure 2.4: A mass mortality of juvenile *Dastilbe elongatus*. Nova Olinda Member of the Crato Formation.

Figure 2.5: Transversely sectioned *Notelops sp.* in typical type 2 concretion (see text for details) from the Romualdo Member. The sediment consists of thin, laterally persistent, alternating carbonate-rich (light bands) and organic-rich (dark bands) laminae. There are repeated cycles in which the importance (i.e. thickness and density) of the carbonate-rich laminae increases. Each cycle is terminated by a sharp return to a thick organic-rich lamina. These cycles are probably linked to seasonal sedimentation and/or seasonal growths of benthic microbial mats.

Scales and phosphatized soft tissues from the upper surface of the fish have collapsed into the fish's body cavity and lie as a geopetal fill (arrowed) on the articulated scales of its lower surface (the weakness across which the concretion split). PRW/9

Figure 2.6: Scour in type 2 concretion from the Romualdo Member. Partially calcified stems and leafs of *Brachyphyllum* sp. ('B'), phosphatized coprolites ('C'), and disarticulated fish bones ('F') are concentrated in a ~6cm deep scour. There is a gradational change in the colour of the concretion from its margins (orange) to its centre (dark grey). This is a weathering phenomenon. PRW/3

2.4



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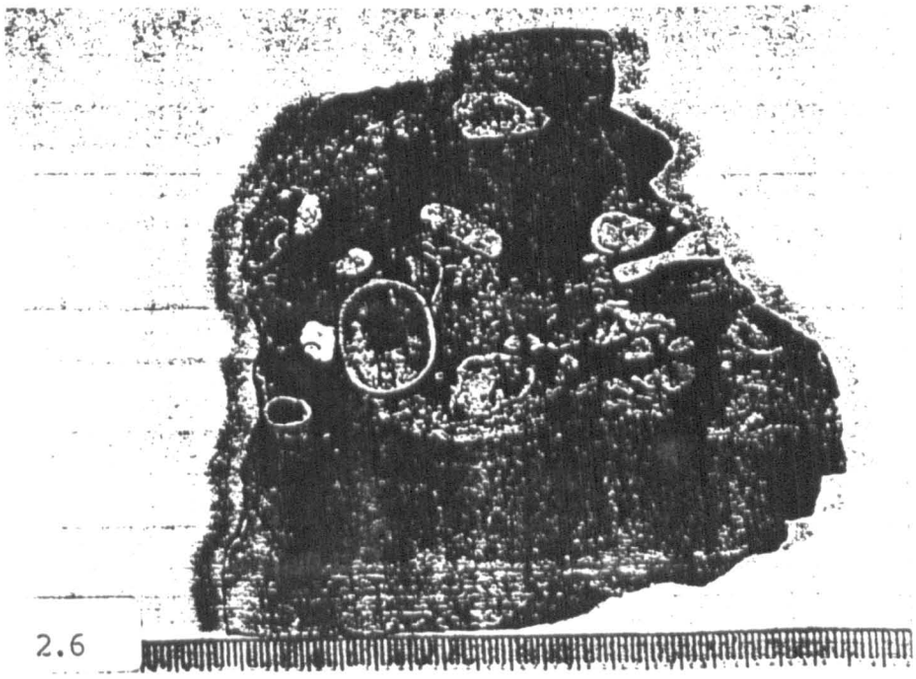


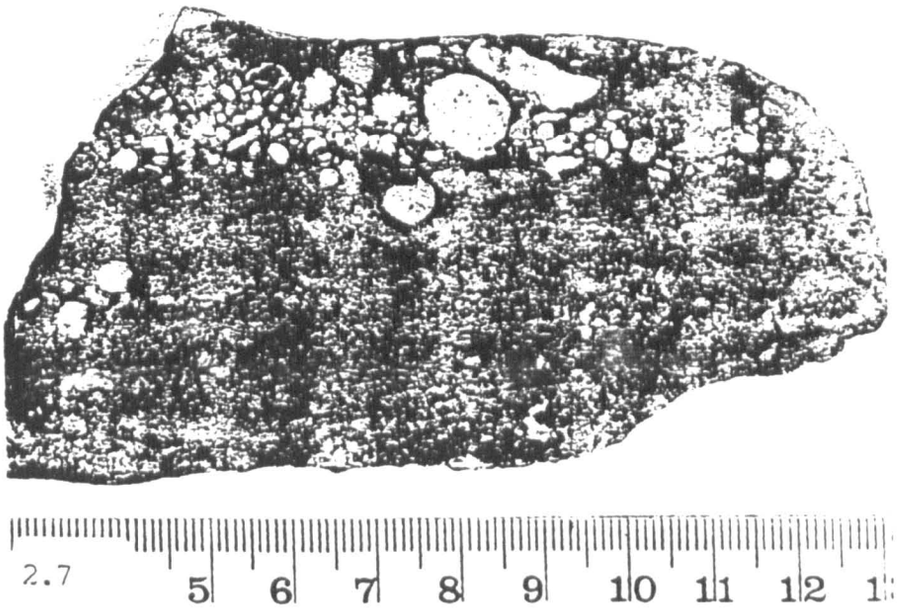
2.6

Figure 2.7: Winnowed lag in type 2 concretion from the Romualdo Member. The lag consists predominantly of imbricated phosphatized coprolites. PRW/18

Figure 2.8: Transversely sectioned *Rhacolepis* sp. in a type 2 concretion of the Romualdo Member. The body cavity of the fish contains over ten generations of concentric non-ferroan calcites. Collapse of the fish's upper surface, and 'plastic' disturbance of the overlying sedimentary laminae preceded the development of the concretion and the precipitation of the internal calcites. The first internal generation of calcite forms a continuous fringe around and over ruptured sections of the fish (arrowed). The last calcites appear to have developed in a liquid or gas filled void formed after the collapse of the carcass. The accumulation of mutually separated skeletal debris and phosphatized soft tissues as a geopetal fill at the base of the carcass ('G') suggests that at the time of calcification, this material was 'floating' in liquified soft tissues. Scale bar in 1cm graduations. PRW/5

Figure 2.9: Transversely sectioned *Rhacolepis* sp. in a type 2 concretion of the Romualdo Member. The fish is enclosed by both an 'early' chalky ferroan calcite concretion, and a 'late' non-ferroan concretion. The collapse of the chalky concretion into the fish indicates it to have formed extremely rapidly around the carcass (at least prior to the collapse of the fish and the release of its putrefaction gases).

Small bones and scales are aligned in the path taken by the escaping putrefaction gases (arrowed). PRW/16



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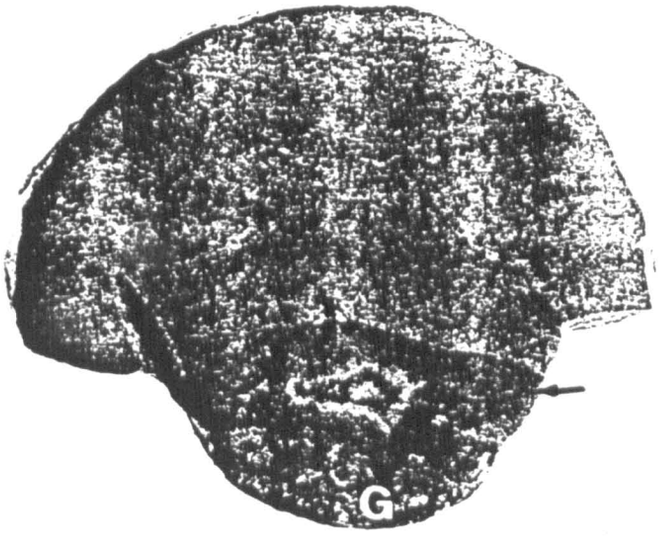

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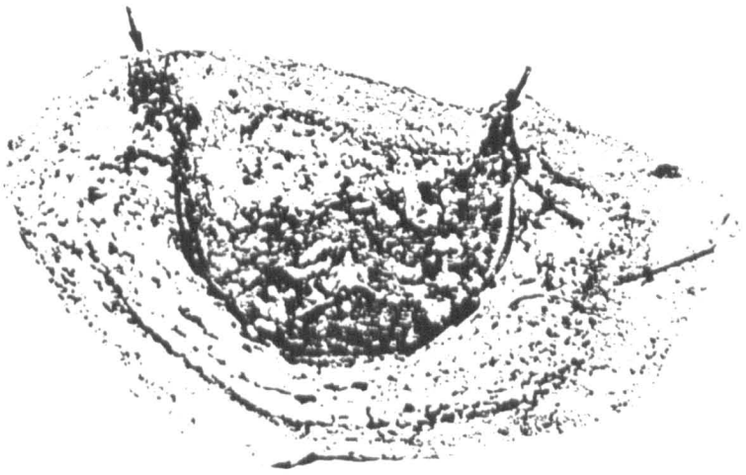


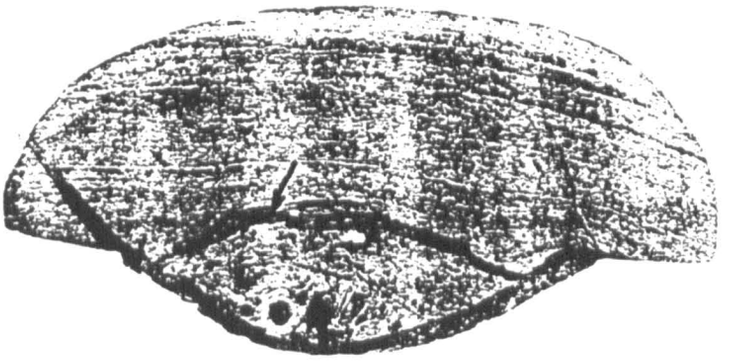
2.9

Figure 2.10: Brittle fracture of laminae (arrowed) above a fish in a type 2 concretion from the Romualdo Member. This suggests the sediment to have been at least partially lithified prior to the collapse of the fish. PRW/6

Figure 2.11: Transversely sectioned *Vinctifer* sp. in a type 2 concretion from the Romualdo Member. Large invaginations of sediment have entered the body cavity from above through small ruptures in the upper body wall. The vertebral column remains fully articulated but is displaced to the lower surface of the fish. Septarian cracks filled with dark brown non-ferroan calcite have developed between adjacent scales (arrowed). DM Santana 15

Figure 2.12: A nearly hollow *Rhacolepis* sp. in a type 2 concretion from the Romualdo Member. The pleural ribs (arrowed) and internal surface of the scales are coated by a series of early calcite generations.
Because of the high angle of repose of the this transgressive fish, the vertebrae and phosphatized soft tissues have become concentrated in the skull. This has left the body cavity largely empty. Lens cap for scale.
Specimen held in the D.N.P.M. at Crato: no identification number.

2.10



2.11

2.12

Figure 2.13: Transversely sectioned *Rhacolepis* sp. in a type 2 concretion from the Romualdo Member. A continuous isopachous, non-ferroan calcite fringes all of the internal skeleton ('C'). This is followed by a dark brown calcite, and a number of discontinuous generations of calcite ('B').

Collapse of the transgressive fish has resulted in articulated sections of dermis ridding over one another to form a series of stacked 'sheets' ('D'). PRW/5

Figure 2.14: Longitudinally sectioned *Rhacolepis* sp. in a type 2 concretion from the Romualdo Member. The body cavity is filled with a complex series of non-ferroan calcites. The last calcite generation forms yellow, subhedral rhombs which project into cavities which were probably liquid or gas filled voids in the decomposing fish ('C').

Sedimentary laminae immediately adjacent to the transgressive fish are down-turned in the direction of the fish's penetration. Laminae surrounding the skull are homogenized in a wedge-shaped zone ('H') anterior to the skull's greatest diameter. The gills ('G') and alimentary ('A') tract are phosphatized. PRW/1

Figure 2.15: SEM. Phosphatized dermis of a *Vinctifer* sp. The tissue's ultrastructural details are completely destroyed by the displacive growth of calcite rhombs. stub 10

2.14

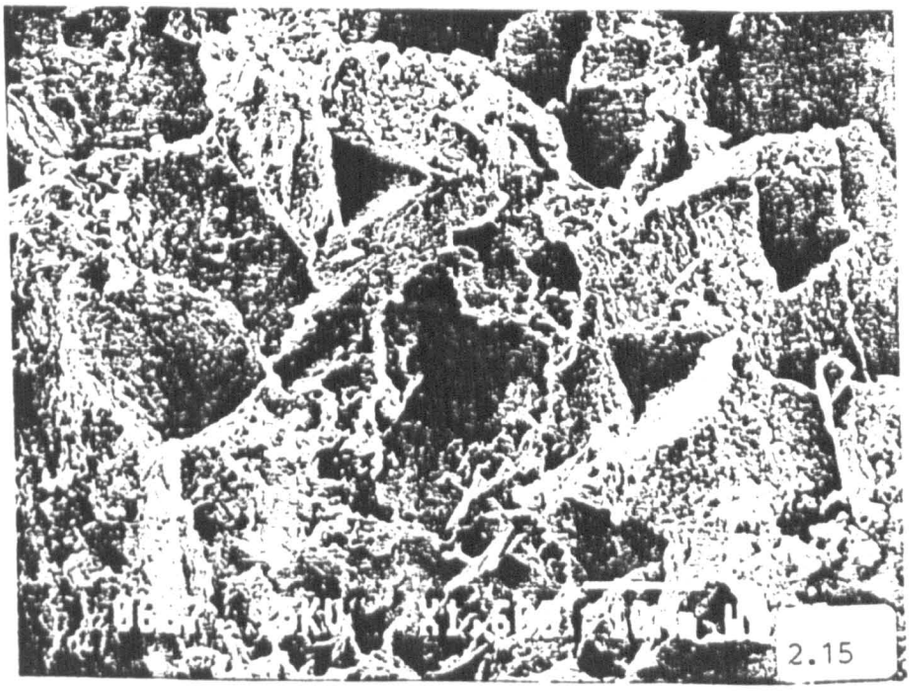
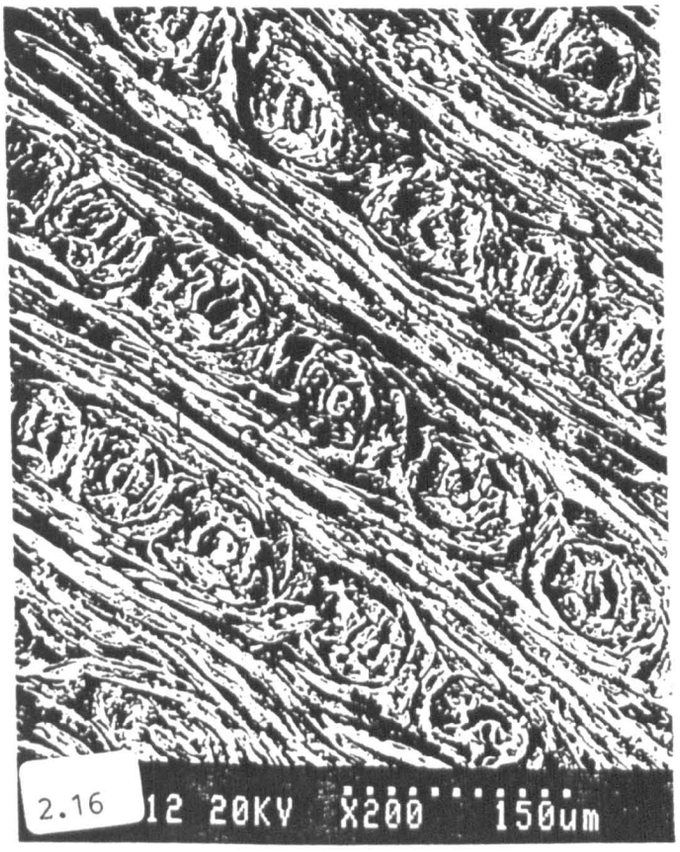


Figure 2.16: SEM. Exceptionally well preserved epidermis of *Brachyphyllum* sp. from a type 2 concretion of the Romualdo Member. Longitudinal cells ('L') and guard cells ('G') surrounding the stomata ('S') are preserved as hollow internal moulds by calcite. stub 9

Figure 2.17: Transverse section of a *Rhacolepis* sp. in a type 2 concretion of the Romualdo Member. The fish has sunk ≈1cm into the sediment. Laminae directly adjacent to the fish are down-warped. Euhedral calcites project into a central void which would originally have been occupied by skeletal muscle and the vertebral column. The alimentary tract is phosphatized (arrowed). PRW/4



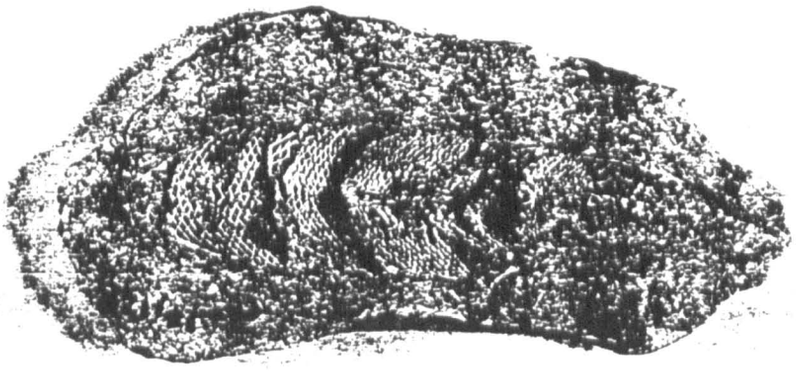
2.17

Figure 2.18: Transverse section of a *Rhacolepis* sp. in a type 2 concretion of the Romualdo Member. The upper surface of the fish has collapsed (arrowed) and permitted the body cavity to become filled with homogeneous sediment. The vertebral column and other internal skeletal elements form a geopetal fill at the base of the fish ('G'). Small corbulid bivalves ('B') are concentrated in the depression created by the collapsed fish. PRW/10

Figure 2.19: The internal surface of the dorsal scales of a *Rhacolepis* sp. in a type 2 concretion from the Romualdo Member. The high angle of repose of this fish in the sediment has prompted articulated sections of its dermis to collapse over one another down the fish's length in response to decay. PRW/13.

Figure 2.20: Transverse section of a *Rhacolepis* sp. in a type 2 concretion from the Romualdo Member. Rotation of the carcass into a more 'ventral-up' position due to shifting pockets of decay gas has resulted in the rotational collapse of the body wall, and the introduction of sediment ('S') into the body cavity. During rotational (anti-clockwise) collapse (arrowed), articulated fragments of dermis ('D') have been forced into the body cavity. The vertebrae and other bones are concentrated as a geopetal fill at the base of the fish ('G'). The alimentary tract is phosphatized ('A'). PRW/8.

2.18



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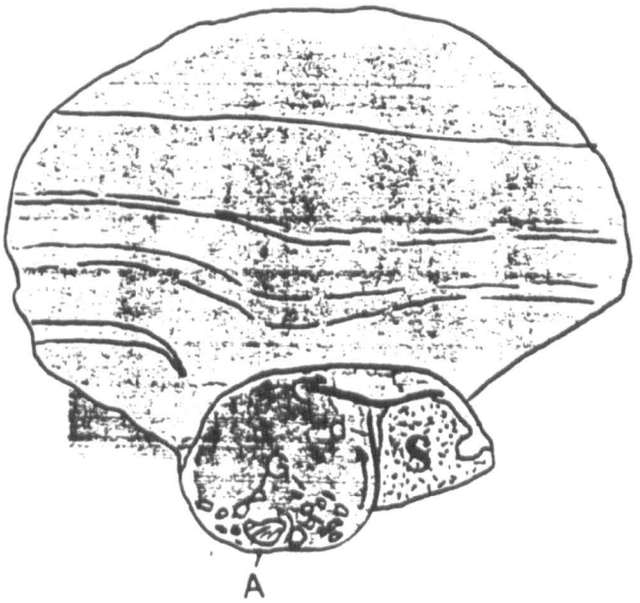
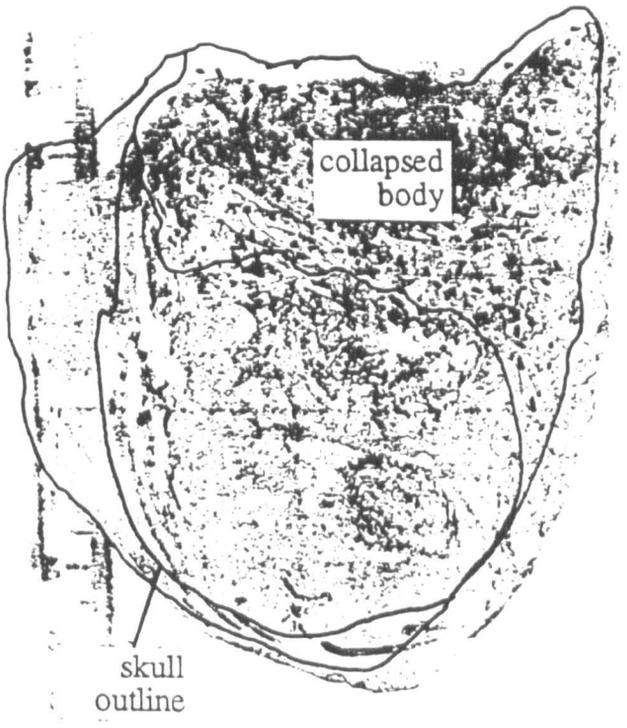


Figure 2.21: Longitudinally sectioned *Lepidotes* sp. in a type 2 concretion from the Romualdo Member. Telescoping was so severe in this near-vertical transgressive fish that its entire body has collapsed into its skull. PRW/23

Figure 2.22: View of the lower surface of a *Notelops* sp. in a type 2 concretion from the Romualdo Member. Postmortem contraction of the caudal fin musclature has resulted in this fin being torn in half and rotated parallel to the caudal peduncle. The skeletal musclature is phosphatized ('M'). PRW/27



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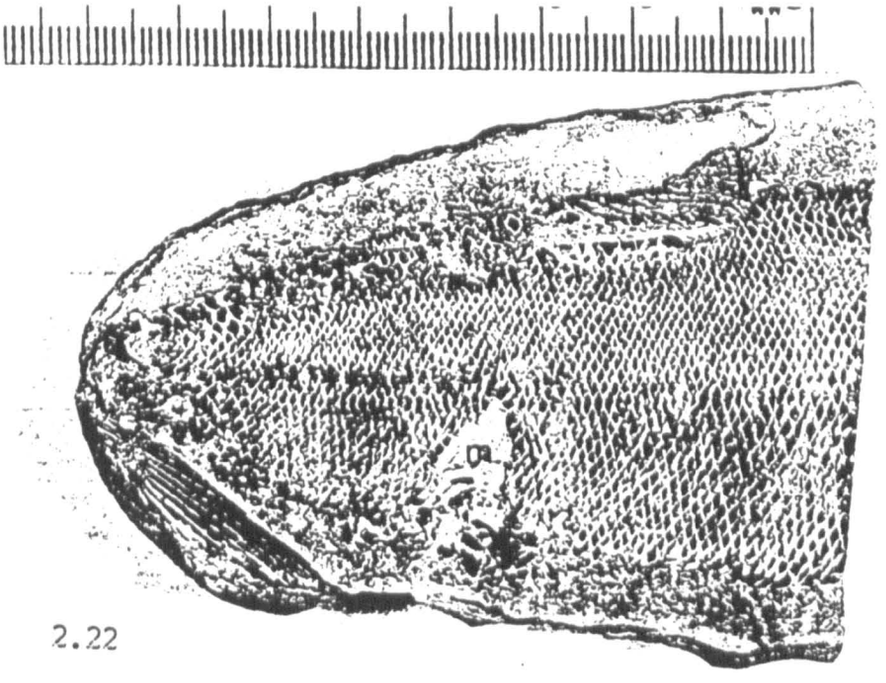
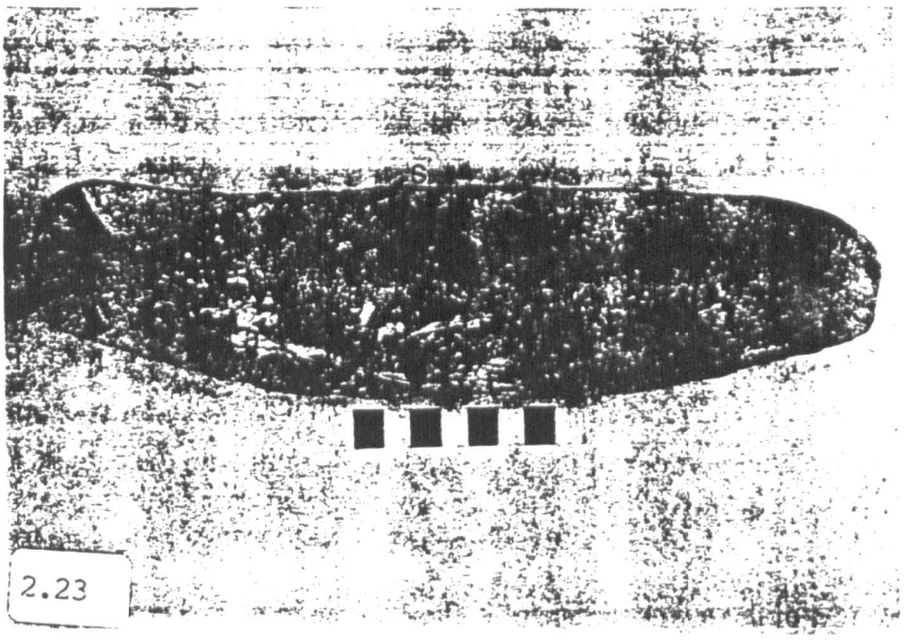


2.22

Figure 2.23: View of the lower surface of a *Notelops* sp. in a type 2 concretion from the Romualdo Member. The scales and phosphatized soft tissues ('S') immediately overlying the body chamber on the fish's upper surface have been 'blown' free from the carcass and lie scattered around on the same bedding plane. This resulted from the violent release of decay gases. Skeletal muscle ('M'), the gas bladder ('G'), and portions of the alimentary tract ('A') are phosphatized. PRW/7

Figure 2.24: View of the lower surface of a *Notelops* sp. in a type 2 concretion from the Romualdo Member. All of the scales, the pelvic-, anal-, dorsal- and pectoral-fins, and most of the pleural ribs are absent. Many of the skull bones, and the skull itself is only loosely attached. The caudal fin however is well preserved. This style of disarticulation is typical of fish carcasses which have experienced a prolonged period of floatation. PRW/2

Figure 2.25: View of the lower surface of a tightly coiled, fully articulated *Vinctifer* sp. in typical type 2 concretion (see text for details) from the Romualdo Member. C.P.C.R. 801. Lens cap for scale.



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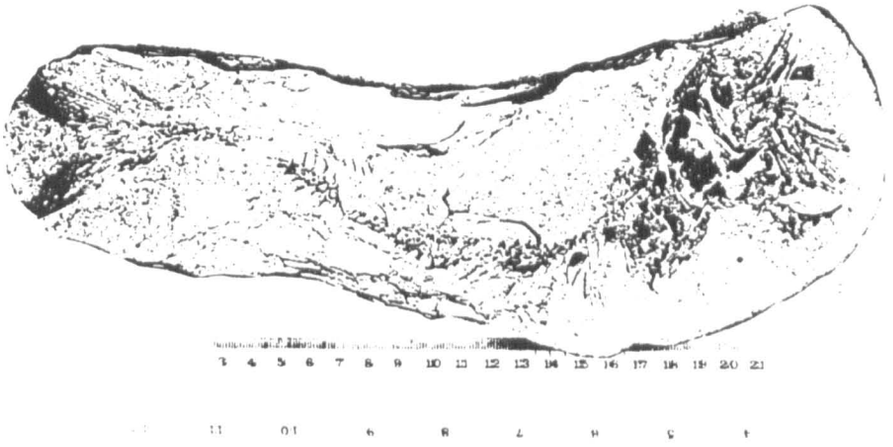

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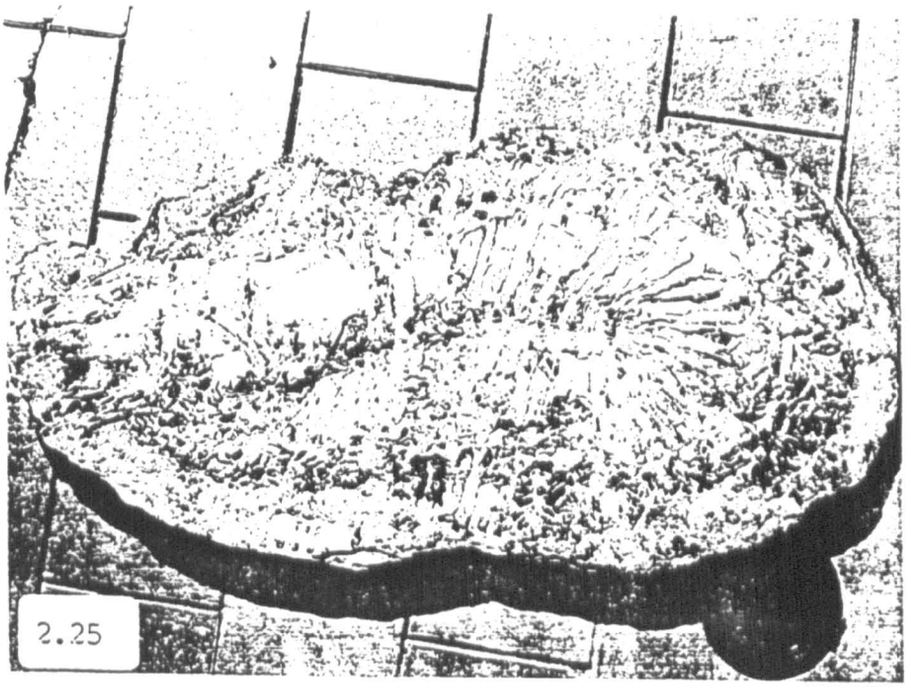
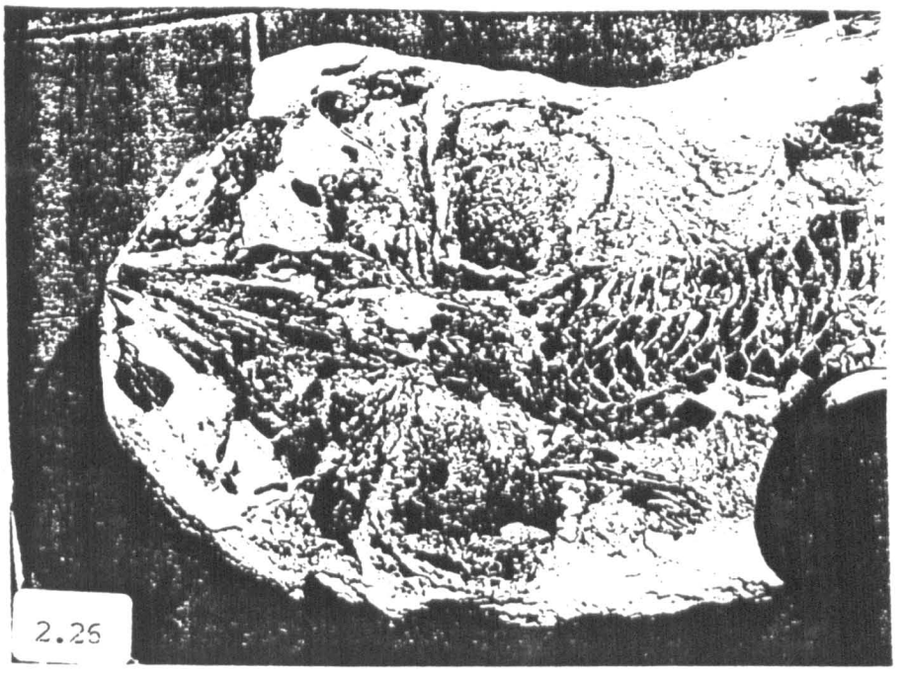


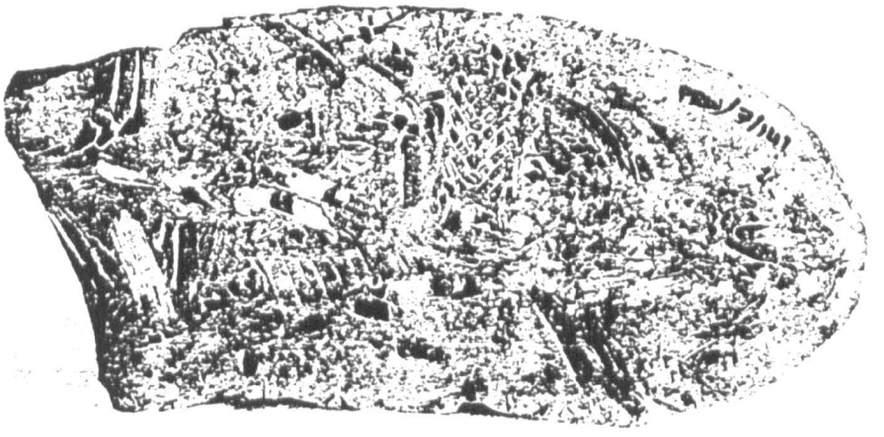
2.25

Figure 2.26: Dorsal view (surface in contact with the sediment surface) of a *Vinctifer* sp. in a type 2 concretion from the Romualdo Member. During decay, the paired bones of the skull fell away symetrically. This indicates the fish to have decayed at the sediment/water interface in stagnant water. Lens cap for scale. Specimen held in the D.N.P.M at Crato: no identification number.

Figure 2.27: Largely dismembered *Vinctifer* sp. in a type 2 concretion from the Romualdo Member. The random distribution of skeletal elements and occurrence of articulated portions of the skeleton is consistent with the carcass having been scavenged. PRW/12

Figure 2.28: View of the lower surface of a *Tharrias* sp. in typical type 1 concretion (see text for detail) from the Romualdo Member. The pectoral and pelvic fins, and the scales surrounding the alimentary tract have been 'blown' away from the carcass but remain associated and partially articulated. This style of preservation is consistent with the explosive release of decomposition gases. C.P.C.R. 2413





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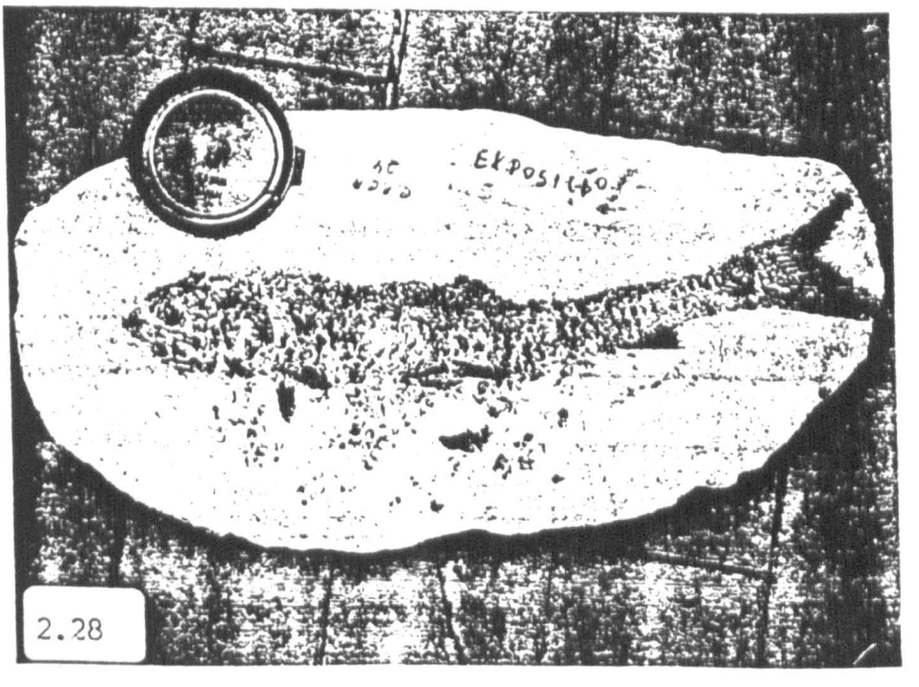
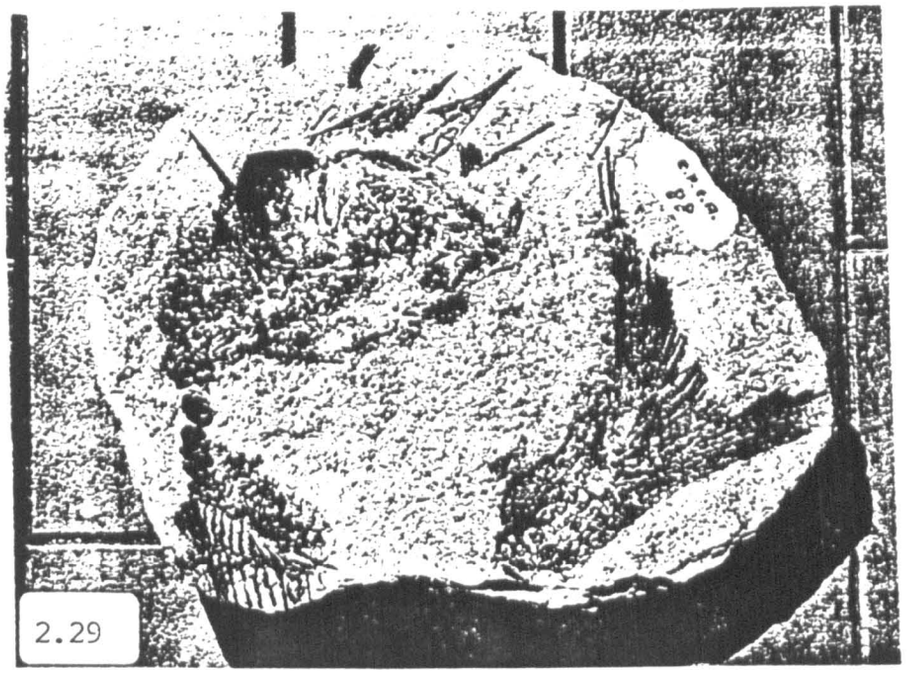


Figure 2.29: View of the lower surface of a *Tharrias* sp. in a type 1 concretion from the Romualdo Member. The body is severely contorted but largely articulated except for the lower jaw bones which are arranged radially around the skull. This style of disarticulation is indicative of fish have experienced partial floatation. C.P.C.R. 88.

Figure 2.30: A type 1 concretion from the Romualdo Member containing an articulated vertebral column of a ≈25cm long fish (?*Tharrias* sp.) against which six fish fry (arrowed) are imbricated. Imbrication of fossils suggests fairly strong currents to have at least periodically existed in the Romualdo Lagoon. PRW/15.



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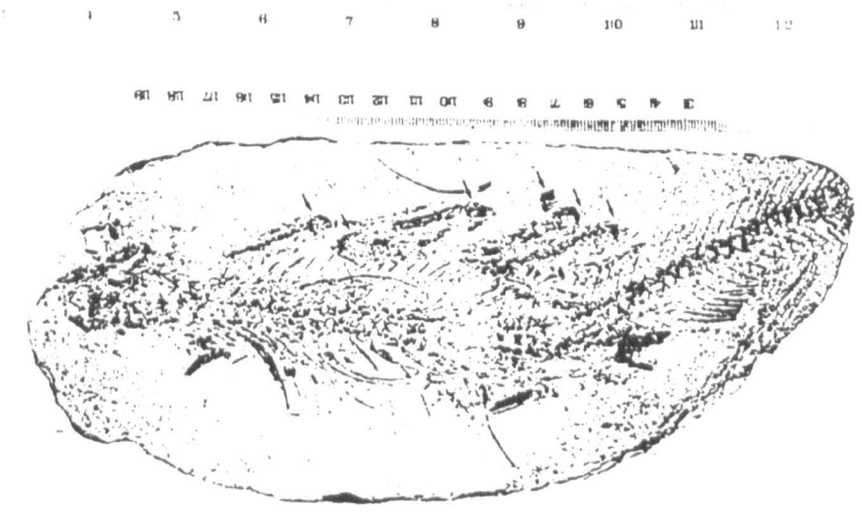
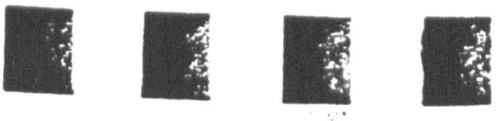


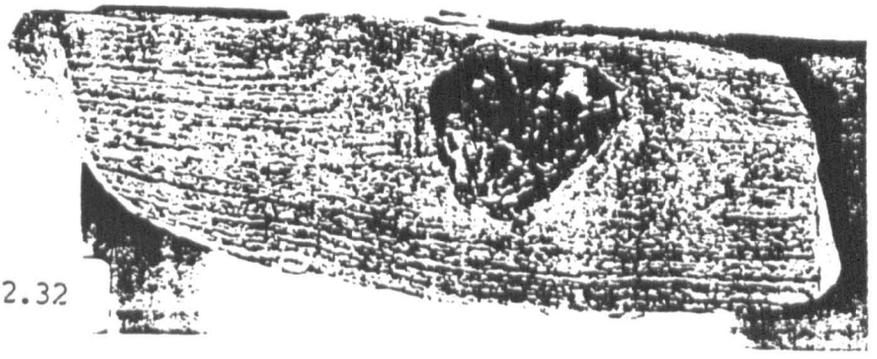
2.30

Figure 2.31: Transgressive *Rhacolepis* sp. in a type 2 concretion from the Romualdo Member. Tetany of the jaws implies death to have been caused by respiratory stress. The body cavity is largely empty suggesting the vertebral column to have collapsed into the skull. The fish has experienced some 'telescoping' whereby articulated segments of scales have slipped over one another down the length of the fish. The concretion is 35cm long. LEIUG 110562.

Figure 2.32: Transverse section through the skull of a bedding-plane normal *Rhacolepis* sp. in type 2 concretion from the Romualdo Member. Sedimentary laminae have been homogenized both above the fish (i.e. where the fish sank through the sediment) and to its side (under undisturbed laminae). This suggests that after sinking into the sediment, the fish thrashed around in the sediment. PRW/4.

2.31



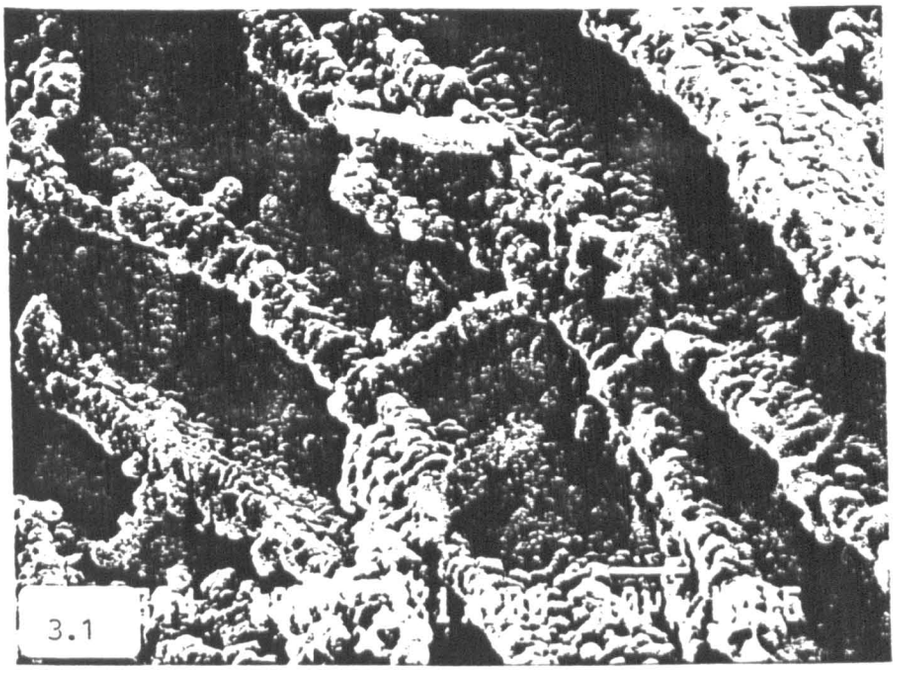


2.32

Figure 3.1: SEM. Morphotype 1a microbes coating secondary gill lamellae of a *Notelops* sp. A 'string' of microbes (arrowed) bridges the gap between two adjacent secondary lamellae. stub 86

Figure 3.2: TEM. Morphotype 1a microbes. The microbes form hollow, frequently interconnected spheres (≈2μm diameter), often with circular, internally skirted apertures (arrowed) and bowl-shaped depressions ('B'). The mineralized envelope consists of an inner layer of small (≈20nm) equidimensional crystallites , and an outer layer of acicular (≈130nm long) crystallites. Micro C5.

Figure 3.3: SEM. Cylindrical, stick-like external moulds of an unidentified micro-organism coexisting with type 1a microbes. stub 27.



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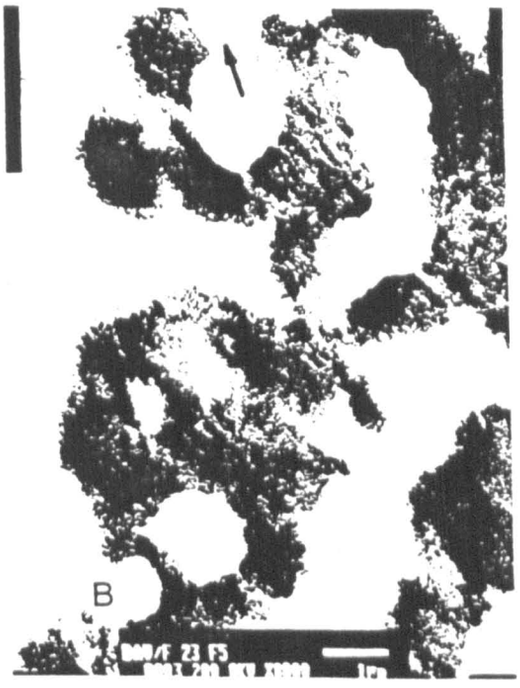
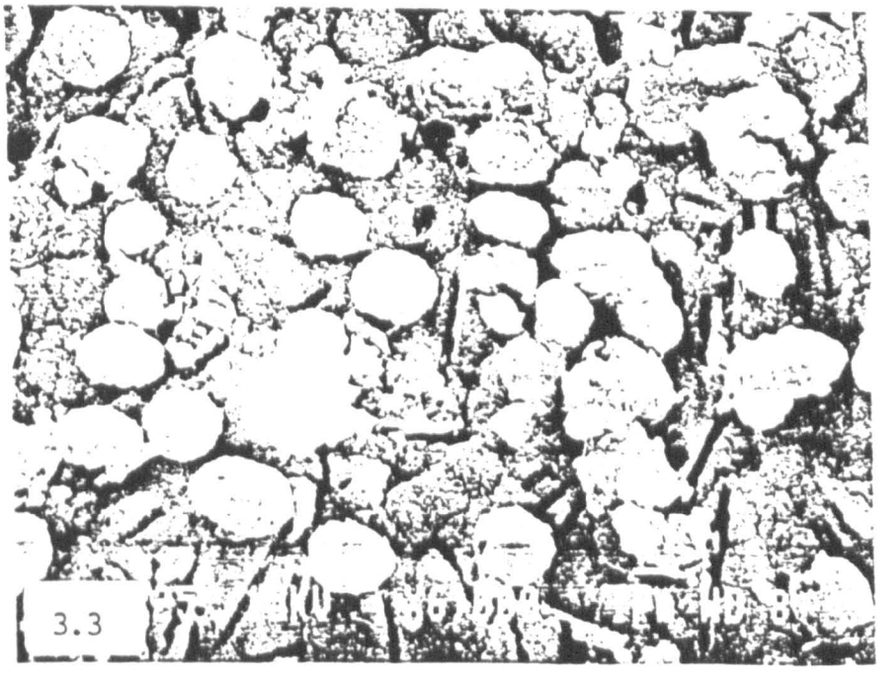


B

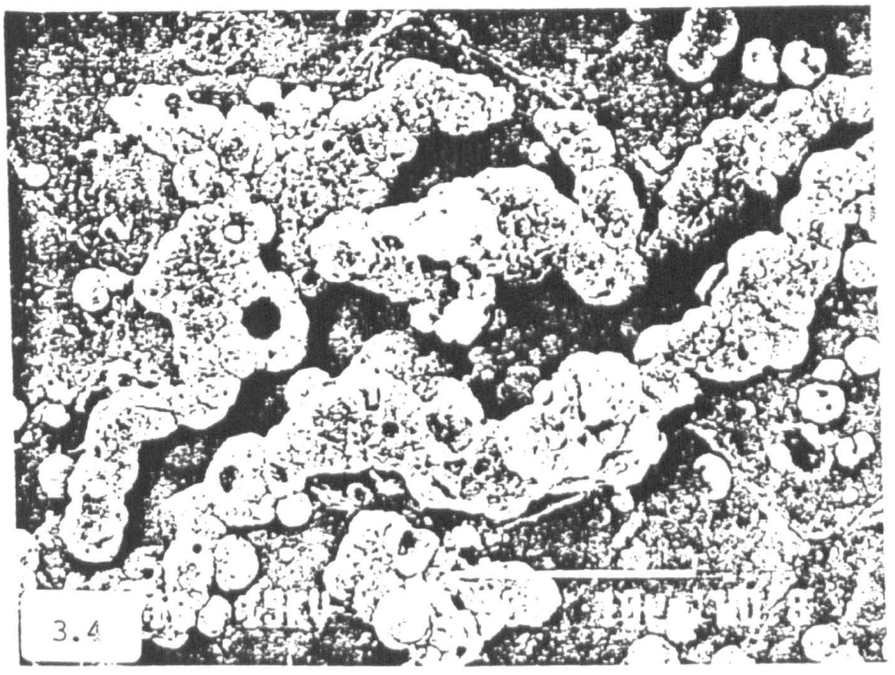
3.2



3.3

Figure 3.4: SEM. Morphotype 1b microbes infesting dermis. The microbes consist of hollow spheres (1.5-3μm diameter) with abundant circular apertures, pits and depressions. Each microbe has a 'cauliflower-like' external surface resulting from the mineralization of well defined 'segments' of the EPS. stub 26

Figure 3.5: SEM. Morphotype 1b microbes infesting the space between adjacent scales ('S') of a *Rhacolepis* sp. Propagation of the microbes results in the development of sinuous strings of the micro-organisms. stub 26.

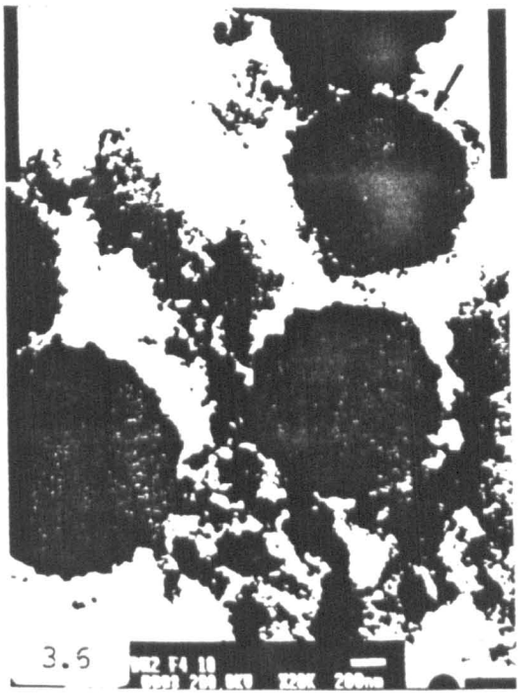

3.4


3.5
007 20KV X1.50K 20.0um

Figure 3.6: TEM. Morphotype 2 microbes. The microbes consist of densely mineralized spherical bodies composed of radially arranged crystallites (≈30nm). The microbe's membrane is occasionally preserved as an un-mineralized space (arrowed). Micro C7.

Figure 3.7: SEM. Morphotype 2 microbes infesting the outer surface of a skeletal muscle fibre. Mutual compression of individual microbes has resulted in the development of a polygonal globular mass. stub 38.

Figure 3.8: SEM. Morphotype 3 microbes replacing the dermis of a *Cladocyclus* sp. This morphotype consists of spherical, hollow bodies (up to 20μm diameter) with a granular external coating. Each sphere probably consists of many microbes. The associated muscle fibres (running E-W) are replaced by inorganic microfabrics. stub 126.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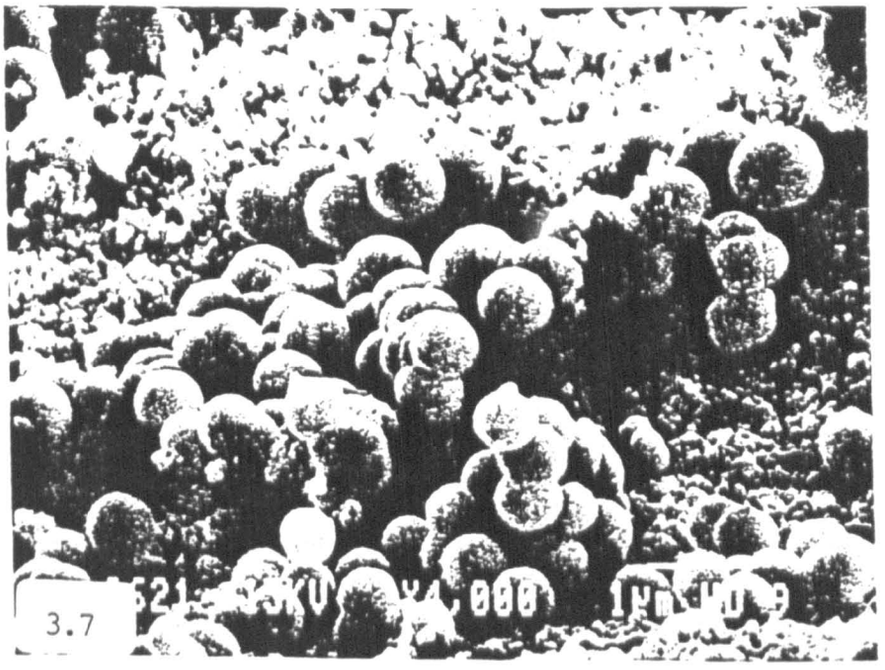
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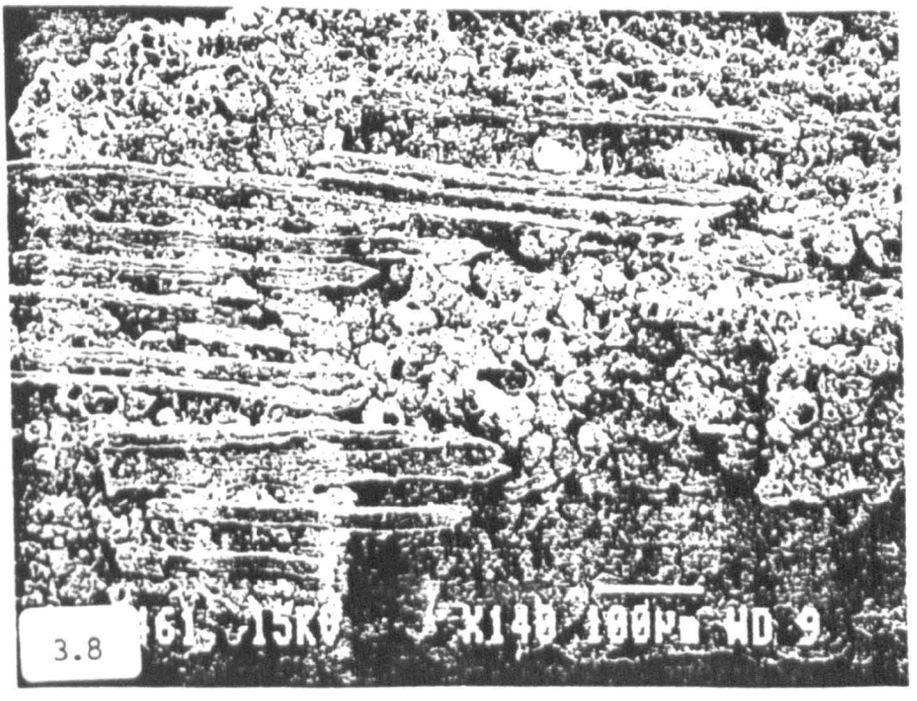
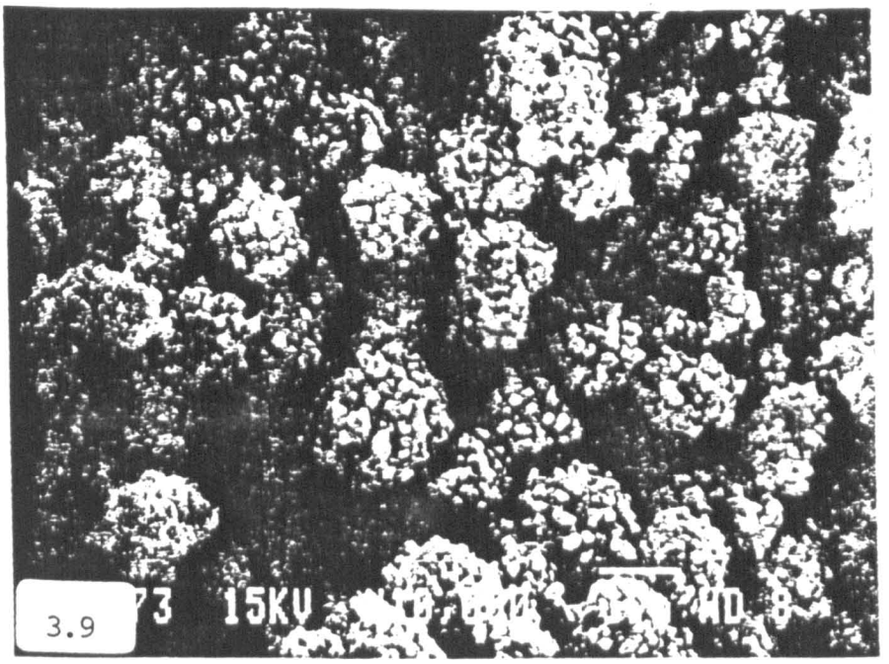

3.8

Figure 3.9: SEM. Morphotype 4 microbes. This morphotype consists of isolated spherical framboids (1-2μm diameter). stub 26.

Figure 3.10: TEM. A single morphotype 4 microbe. This microbe is hollow with a number of mineralized protrusions extending from its external surface. Each protrusion consists of a mass of densely packed, radiating crystallites (50nm long). Micro C9.

Figure 3.11: TEM. Morphotype 4 microbes replacing skeletal fish muscle. The microbes are in contact with one another but are not interconnected and do not mutually compress one another. Many contain one or two concentrically located spheres each separated from the other by a thin un-mineralized gap (a membrane). These probably represent mineralized subcellular bodies. Micro D2.

Figure 3.12: SEM. Morphotype 5 microbes. This morphotype consists of densely mineralized spheres (up to 4μm diameter) which form interconnected, polyhedral, globular masses. Each microbe is preserved by both a solid internal mould, and a thin (500nm) external coating. These are separated by a 1μm gap representing the microbes membrane. Many of the microbes are perforated by a small aperture (arrowed). stub 18


3.9
15KV


3.10
X10K 500nm


3.11

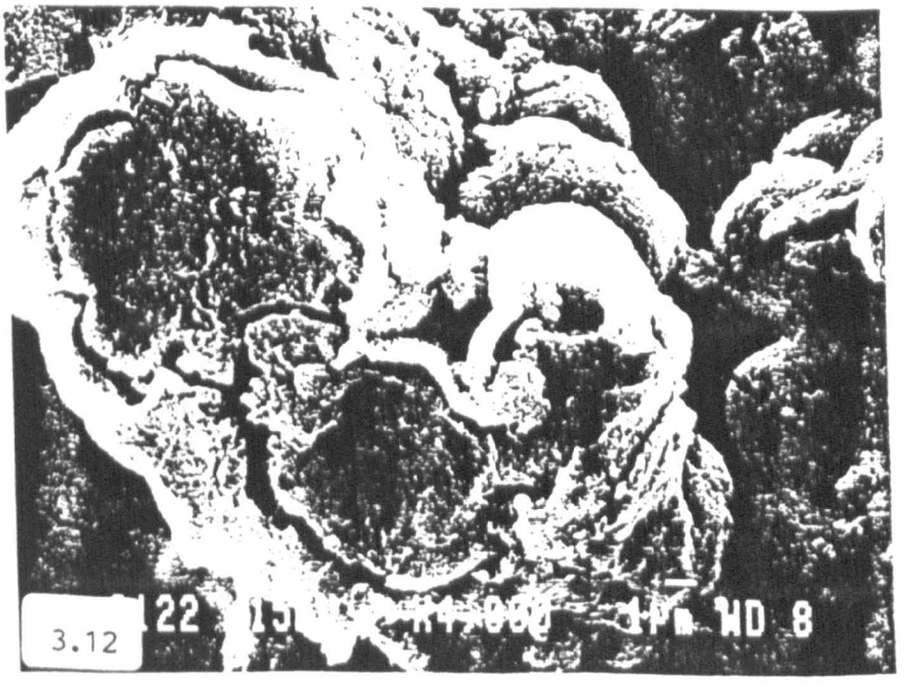

3.12
WD 8

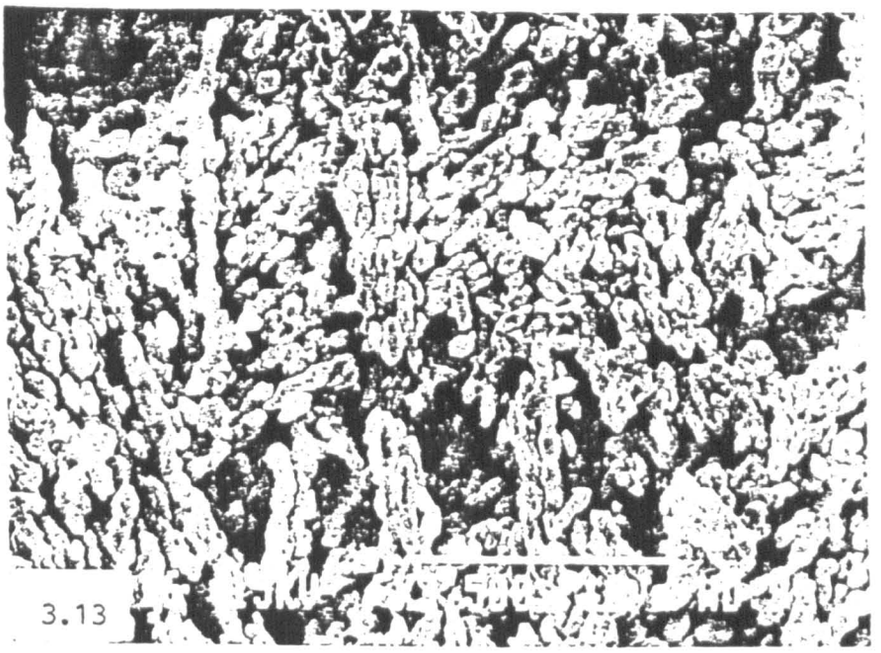
Figure 3.13: SEM. Morphotype 6 microbes pseudomorphing smooth muscle fibres in the muscularis externa of the digestive tract of a *Rhacolepis* sp. The rod-shaped microbes are preserved as a solid internal mould. They quite accurately reproduce the substrate, and each microbe is directly in contact with two other individuals. stub 142.

Figure 3.14: TEM. Gas vesicles in a large coprolite. The diameter of the vesicles varies enormously but all are preserved as external moulds by microgranular apatite. Each vesicle is separated from its neighbours by a mineralized wall. There has been some compression of the coprolite resulting in the elongation of the vesicles in one direction (NE-SW). Micro D4.

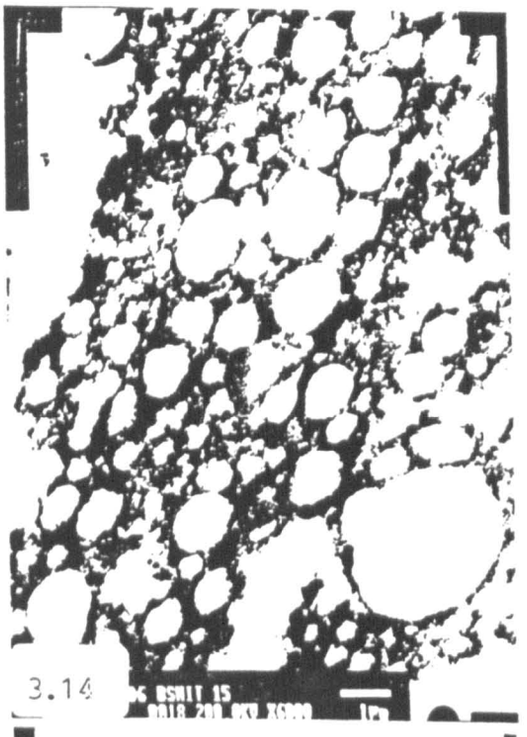
Figure 3.15: TEM. Type 1 inorganic microspheres. These microspheres consist of irregular, sub-spherical bodies (~600nm diameter) composed of acicular crystallites which radiate from a hollow core. Adjacent microspheres frequently impinge on one another. Micro D6.

Figure 3.16: TEM. A type 2 inorganic microsphere. These microspheres are subspherical bodies (1μm diameter) composed of randomly orientated acicular crystallites (100nm long). Micro D8.

Figure 3.17: TEM. Type 3 inorganic microspheres. These microspheres are nearly perfectly spherical masses (600nm-1μm diameter) composed of densely packed radiating crystallites (~100nm long). The microspheres often coalesce. Micro D10.



3.13



3.14

3.15

3.16

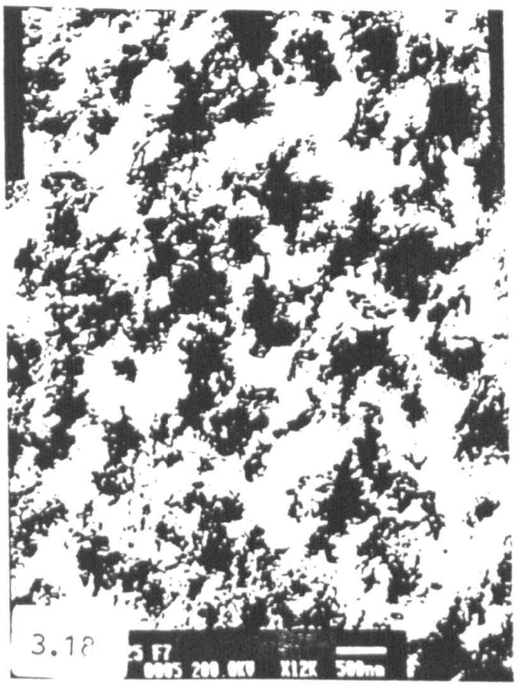
3.17

Figure 3.18: TEM. Type 4 inorganic microspheres. The microspheres are rather irregular masses (up to 800nm diameter) composed of a fine grained core (crystallites <20nm) from which acicular crystallites (200nm long) protrude. Adjacent microspheres frequently coalesce. Micro E1

Figure 3.19: TEM. Inorganic non-spherulitic (microgranular) apatite consisting of crystallites ≈30nm in length. Micro E3.

Figure 3.20: SEM. A blood cell replaced by inorganic microgranular apatite. Destroyed.

Figure 3.21: SEM. ?Blood cell replaced by inorganic microspheres. stub 212.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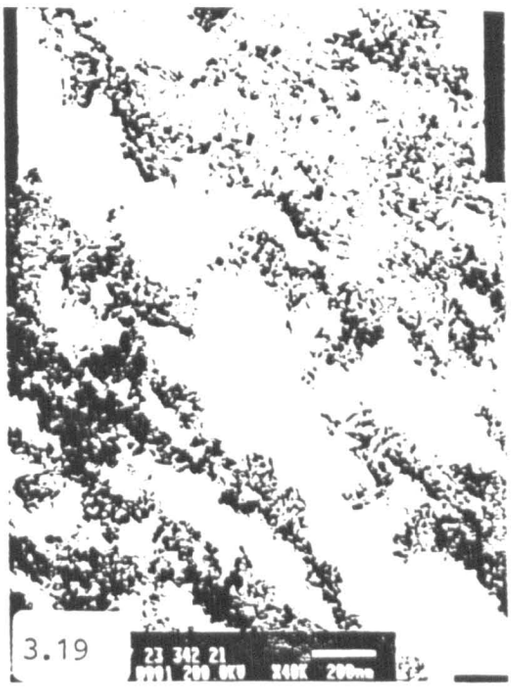
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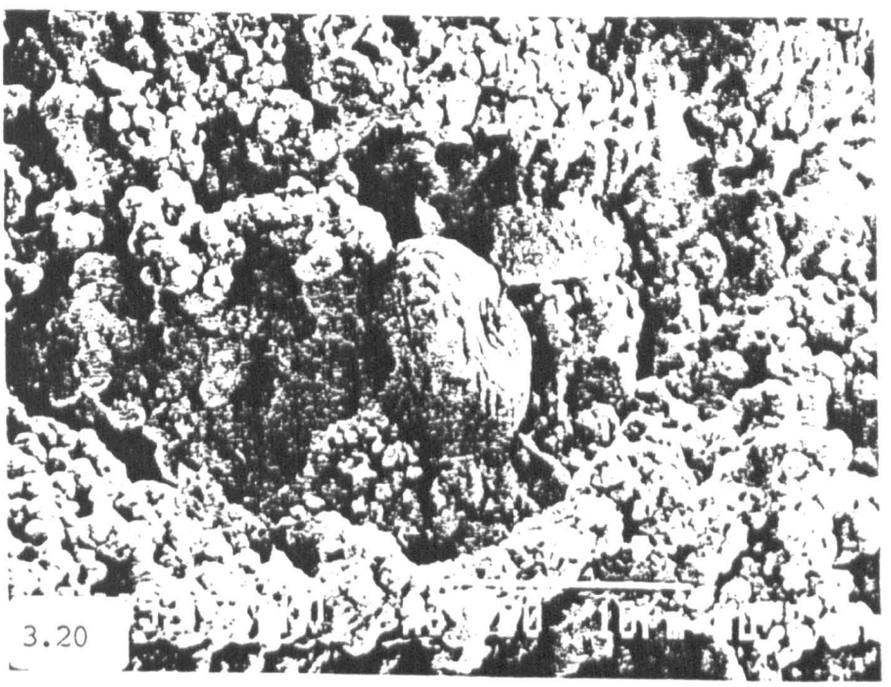
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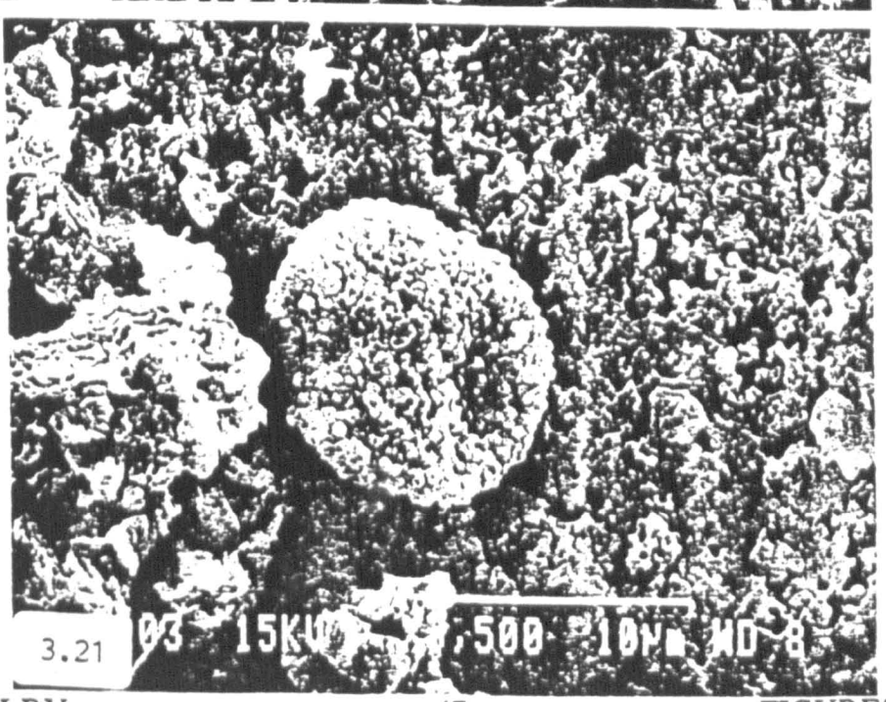

3.21

Figure 4.1: **A partially acid digested *Rhacolepis* sp. with some soft tissues still *in situ*. The vertebral column ('V'), stomach contents ('S'), dermis ('D') and gill apparatus ('G') are clearly visible. LEIUG 107851. Centimetre scale.**

a) Typical SEM image of the dermis of *Rhacolepis* sp. consisting of randomly orientated collagenous fibres. stub 27.

b) Typical SEM image of part of the gill apparatus of *Rhacolepis* sp. and *Notelops* sp. Three gill filaments ('F') and their respective secondary lamellae are figured.

c) Typical SEM image of a portion of the alimentary tract of a *Notelops* sp. The lumen ('L') is tightly constricted. stub 135.

d) Typical SEM image of skeletal muscle fibres of *Rhacolepis* sp. Each fibre is clearly banded. stub 79.

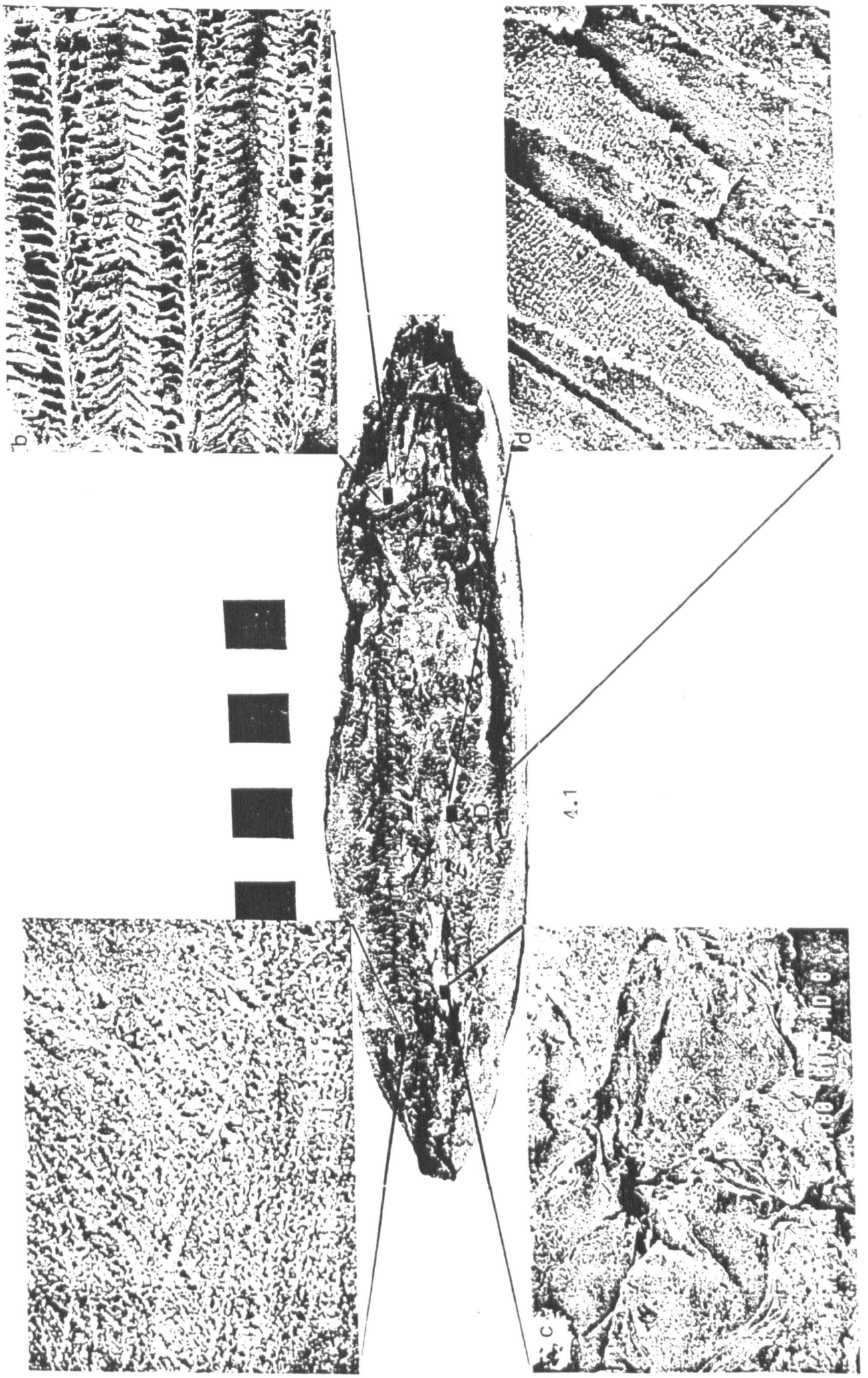
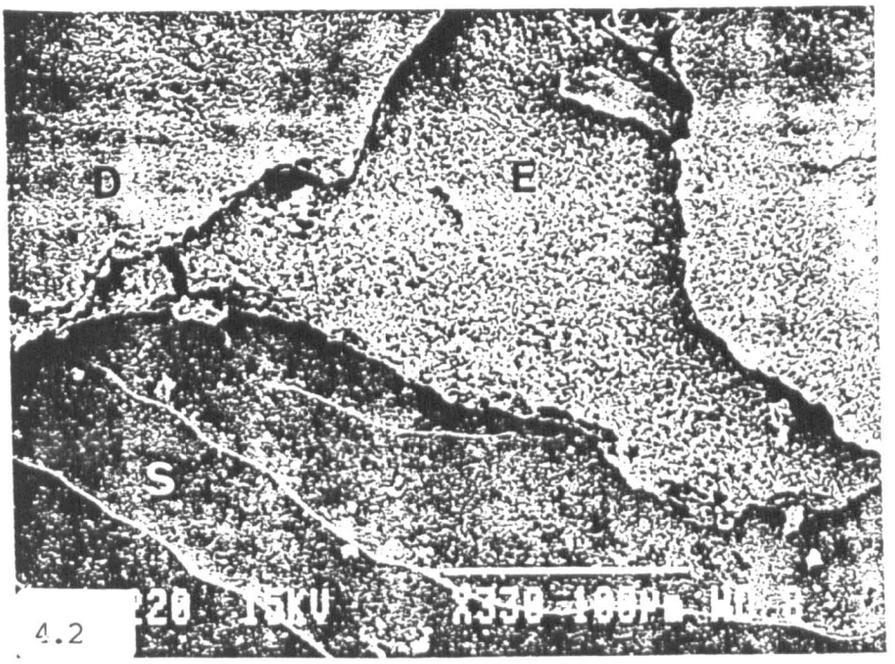

b
d
4.1
c

Figure 4.2: SEM. Phosphatized fish skin. Two distinct layers may be distinguished lying on the scale ('S'). The epidermis ('E') consists of cells packed densely in anastomosing collagenous plasma membrane tonofibrils. The dermis ('D') consists of a number of superimposed layers of collagen and elastic fibres. stub 27

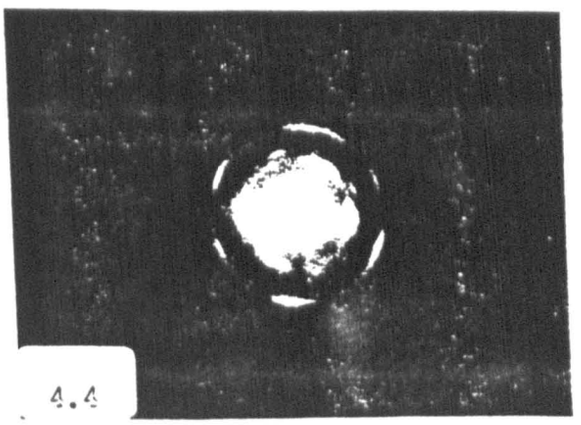
Figure 4.3: TEM. Exceptionally well preserved fusiform layer of fish epidermis. Cells ('C') are 'packed' in a dense 'mat' of collagenous plasma membrane tonofibrils. The cells themselves are not mineralized. Some however, contain a densely mineralized mass which may be an organelle ('O'). The plasma membrane tonofibrils are heavily mineralized by microgranular apatite. Three chemically isolated compartments existed during mineralization: the extracellular space, the cytosol, and the organelles. Micro E5.

Figure 4.4: TEM. Micro-electron diffraction pattern of the phosphatized plasma membrane tonofibrils of the fusiform layer of fish epidermis. There are clear groupings of spots which imply the crystallites of this tissue to have a preferential alignment. Micro E5.



4.2

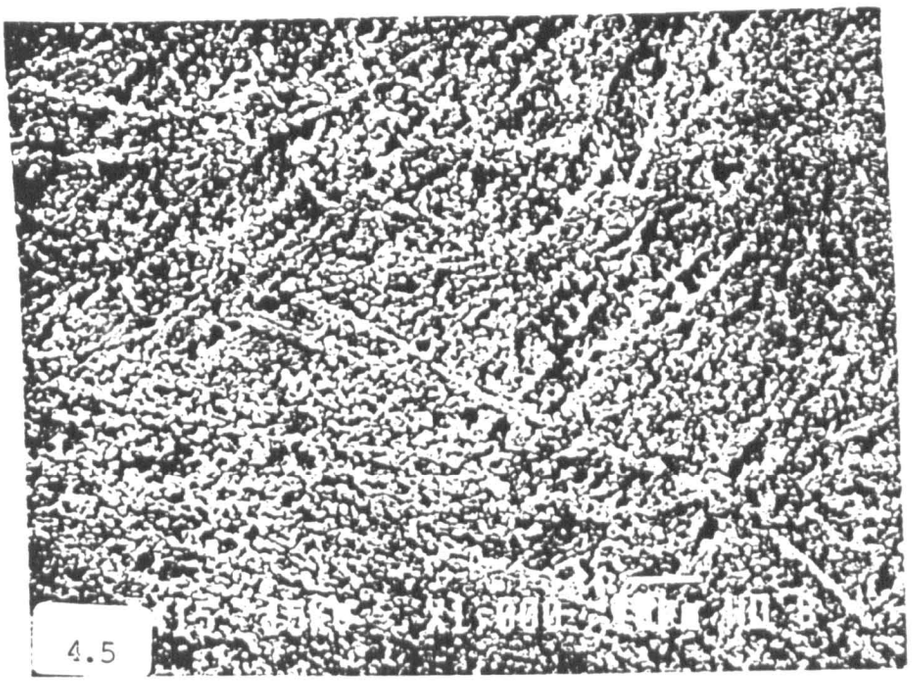
4.3



4.4

Figure 4.5: SEM. Stratum compactum of fish dermis. The stratum compactum consists of a number of superimposed layers of mutually aligned collagen and elastic filaments replaced by inorganic microspheres. stub 27.

Figure 4.6: SEM. Fish hypodermis. This consists of randomly orientated bands of fibrous connective tissue. stub 20.

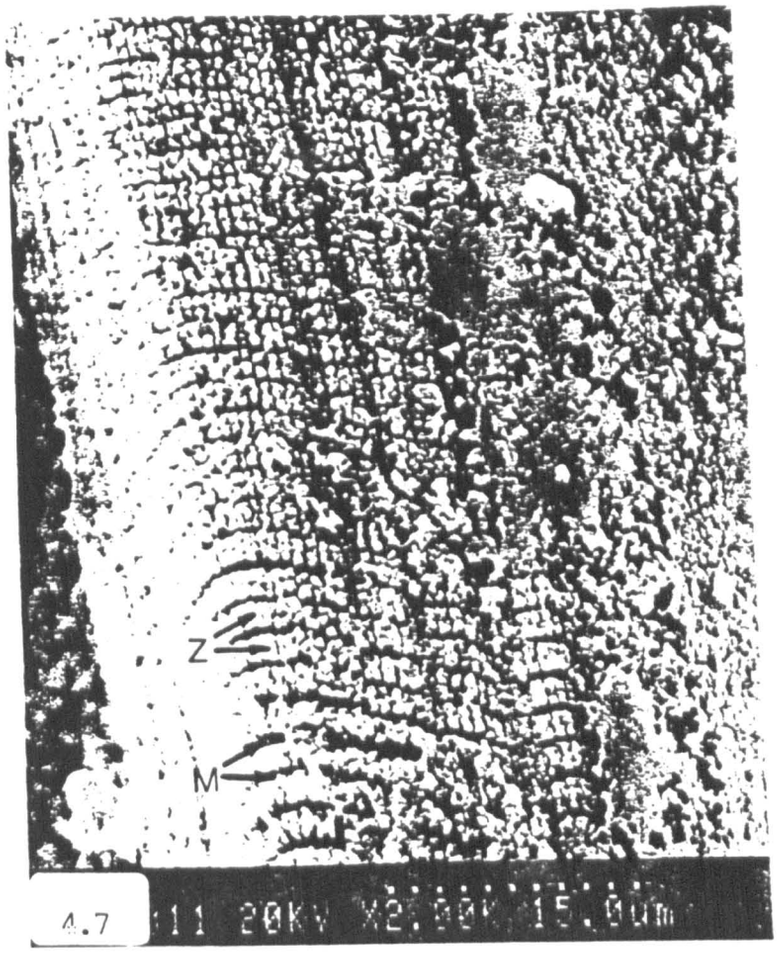


4.5

4.6

Figure 4.7: SEM. Skeletal muscle fibre of a fish replaced in exceptional detail by microgranular apatite. The longitudinal bands are myofibrils. Each myofibril is divided along its length into cuboidal couplets, each about a micron in length. The un-mineralized gaps between each set of couplets along the length of the myofibrils are the M-lines ('M'). Each couplet is bisected by a thin un-mineralized zone (<100nm thick) which corresponds to the Z-disc ('Z'). Each couplet therefore consists of a Z-disc and two halves of adjacent sarcomeres. stub destroyed.

Figure 4.8: SEM. The terminal ends of striated muscle fibres replaced by inorganic microspheres. The simultaneous development of M-lines with enlarged widths (a taphonomic phenomenon) along the entire length of the fibres results in the development of a series of 'stacked sheets' consisting of laterally connected taphonomically damaged sarcomeres. Scars (arrowed) running longitudinally along one fibre correspond to the point of entry into the fibre of capillaries. stub 145.


Z
M
4.7

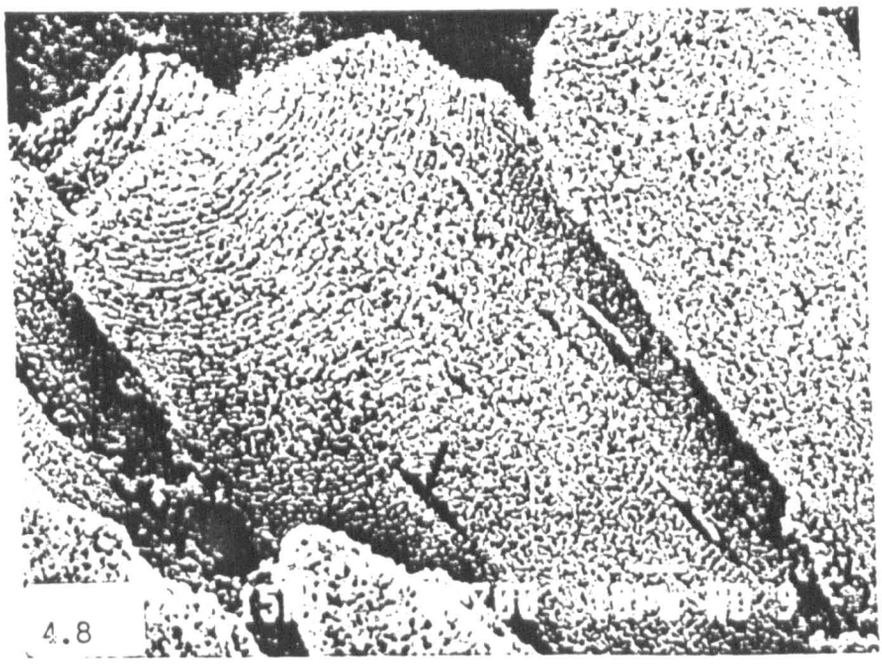
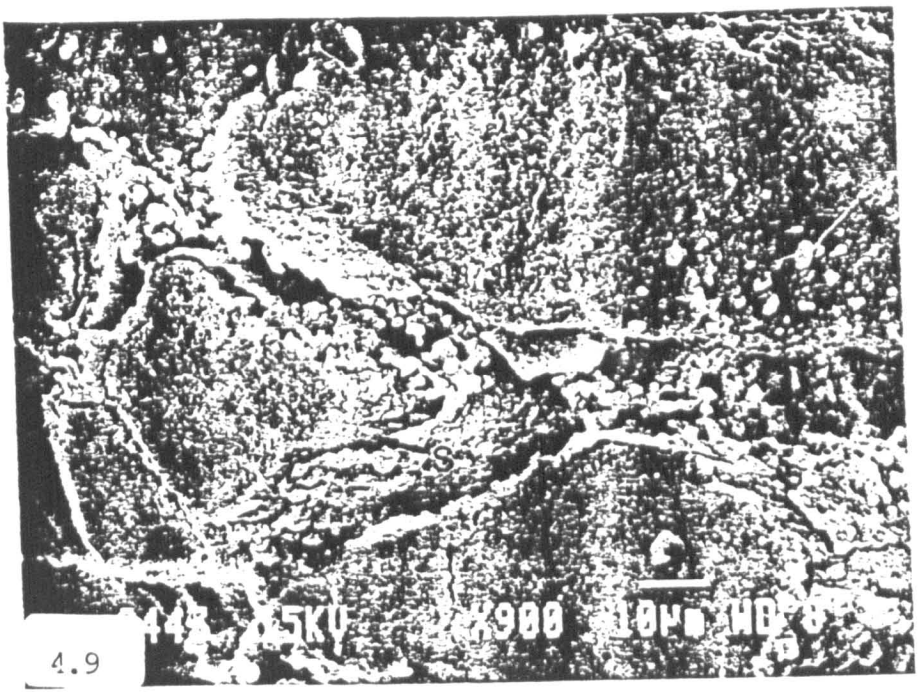

4.8

Figure 4.9: SEM. Terminal ends of skeletal muscle fibres. The sarcolemas are preserved as un-mineralized gaps ('S') between the inorganically mineralized muscle fibres and a coating of mineralized microbes. The microbes completely obscure the ultrastructure of the fibres. stub 54.

Figure 4.10: SEM. A skeletal muscle fibre in which the banding is partially obscured by the deposition of a thin (≈100nm) layer of microgranular apatite between the fibre proper and the the overlying sarcolema (not preserved). A nucleus ('N') is preserved as an internal mould by microgranular apatite. stub destroyed.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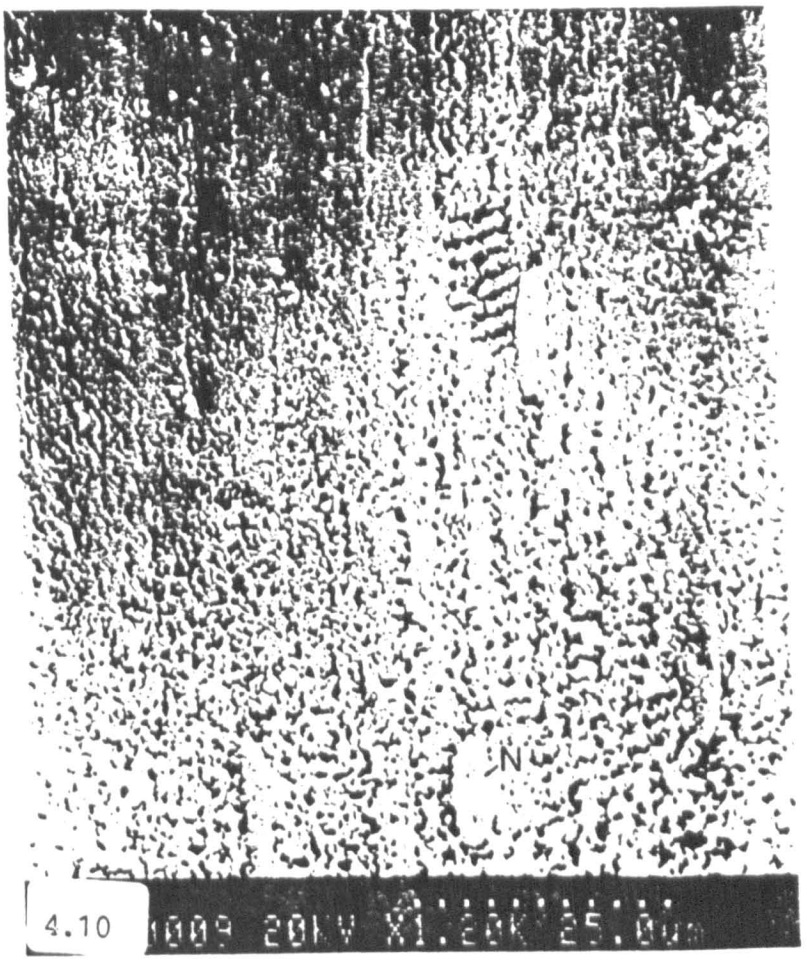
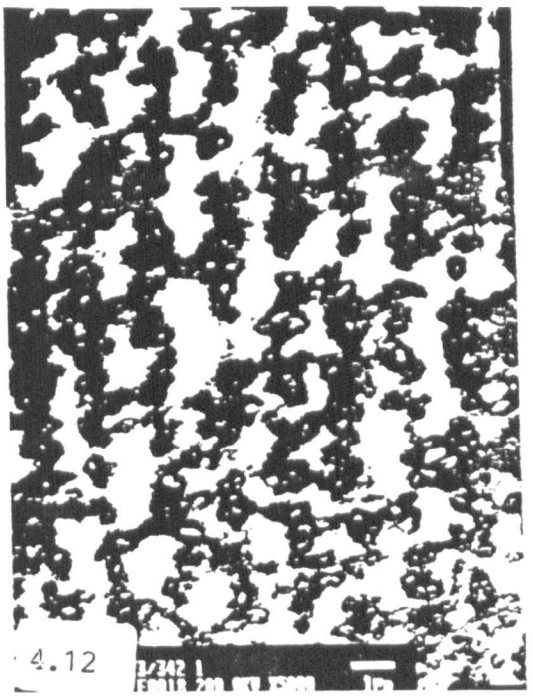

4.10

Figure 4.11: TEM. Two striated muscle fibres replaced by inorganic microspheres. Their sarcolemas ('S') are preserved by partial external coatings. Micro C3.

Figure 4.12: TEM. Transverse banding in a skeletal muscle fibre replaced by type 1 inorganic microspheres. Micro D6.

Figure 4.13: SEM. Skeletal muscle fibre and nucleus ('N') replaced by inorganic microspheres. The myofibrils are well preserved and run NW-SE. Each half of every sarcomere is replaced by an inorganic microsphere.
The nucleus has an irregular outline and rather deflated appearance indicative of the advanced stages of decay. Destroyed.


S
4.11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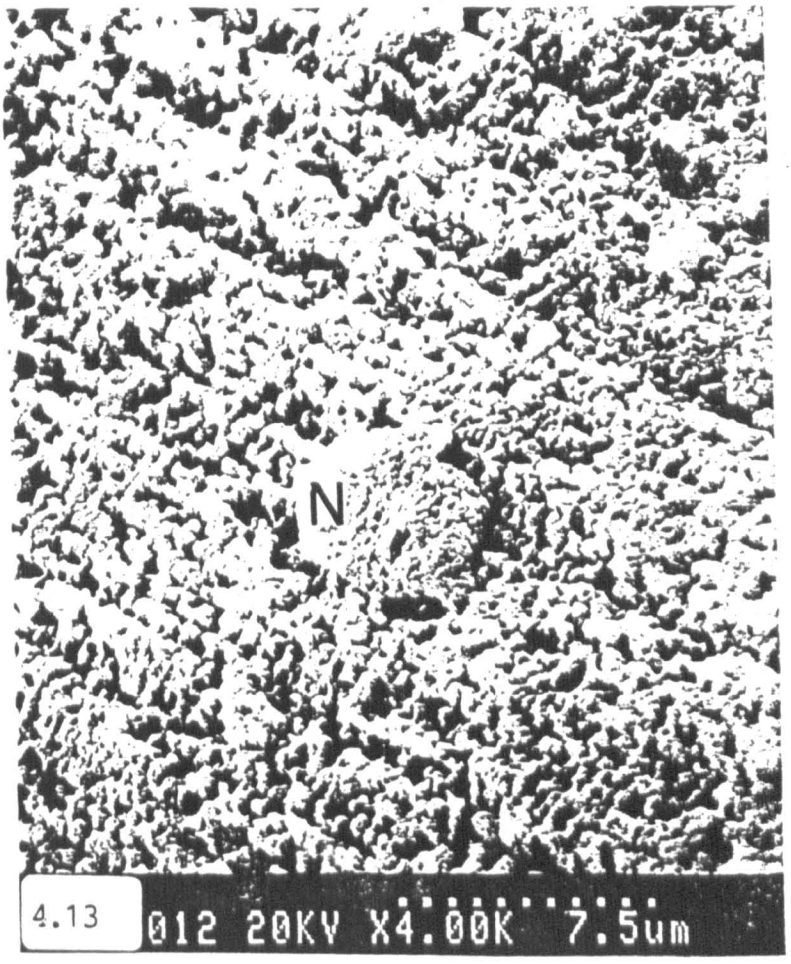
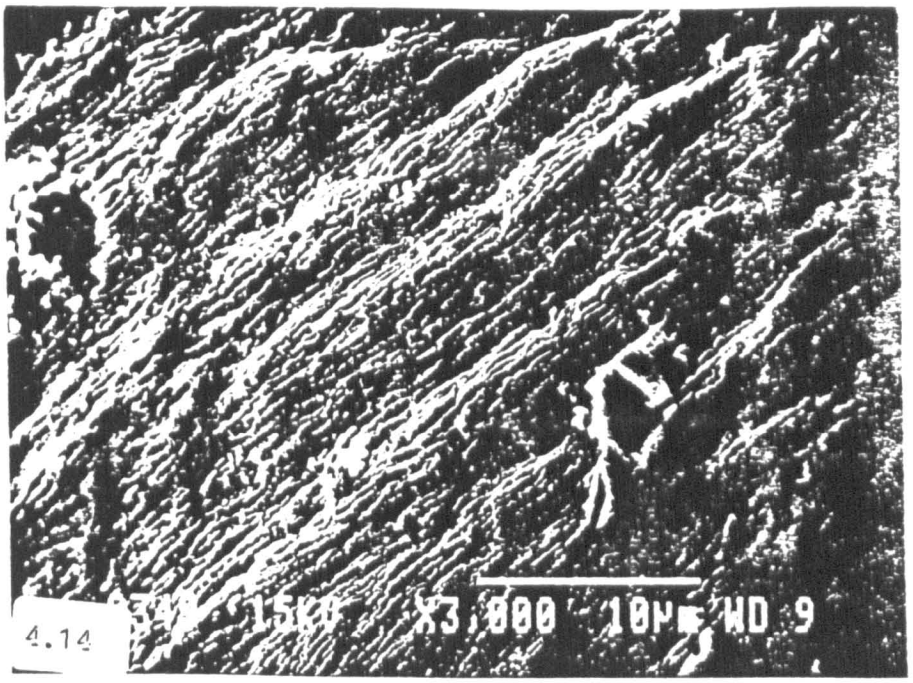
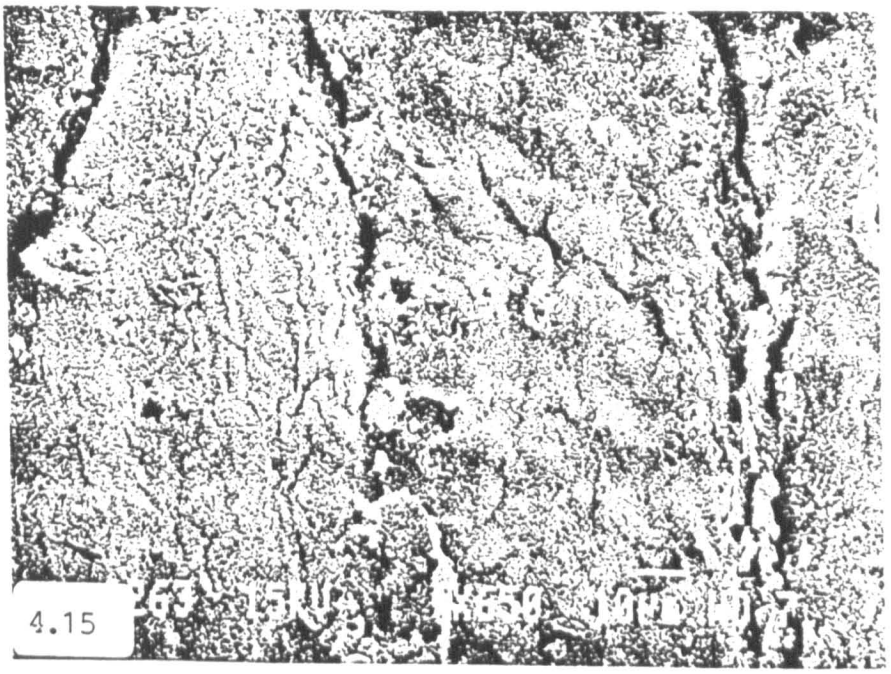

N
4.13
012 20KV X4.00K 7.5um

Figure 4.14: SEM. Connective fibrils protruding from the terminal ends of a striated muscle fibre. Every fibril is replaced by microgranular apatite and displays a regular periodicity of thickenings which probably correspond to a repetitive structural unit in the fibrils. Destroyed.

Figure 4.15: SEM. The sarcolemas of two striated muscle fibres replaced by microgranular apatite. The irregular folds in the sarcolemas are taphonomic phenomena caused by the collapse of the fibres. stub 79.

Figure 4.16: TEM. Two striated muscle fibres replaced by microgranular apatite. The sarcolema ('S') of one of the fibres is preserved by an external coating of microgranular apatite. The sarcolema itself is not mineralized. Capillaries ('C') are also preserved but remain un-mineralized. Micro E7.


4.14
X3,000 10μm WD 9


4.15


4.16

Figure 4.17: SEM. Elliptical nuclei on the peripheral surface of a striated muscle fibre. The nuclei are arranged in distinct rows with their long axis parallel to that of the fibre. Both the fibre and the nuclei are replaced by microgranular apatite. One of the nuclei (arrowed) displays evidence of partial collapse. Banding is well preserved in the fibre. stub destroyed.

Figure 4.18: SEM. Two striated muscle fibres replaced by microgranular apatite. The nuclei ('N') of one fibre are preserved as 'scars' which correspond to their former point of attachment to the fibre. The nuclei appear to have protected the underlying fibre from being coated with microgranular apatite but were themselves not phosphatized. Sections of the system of T-tubules ('T') are preserved as internal moulds on the surface of both muscle fibres. stub 78.


4.17
033 20KV X1.50K 20.0um

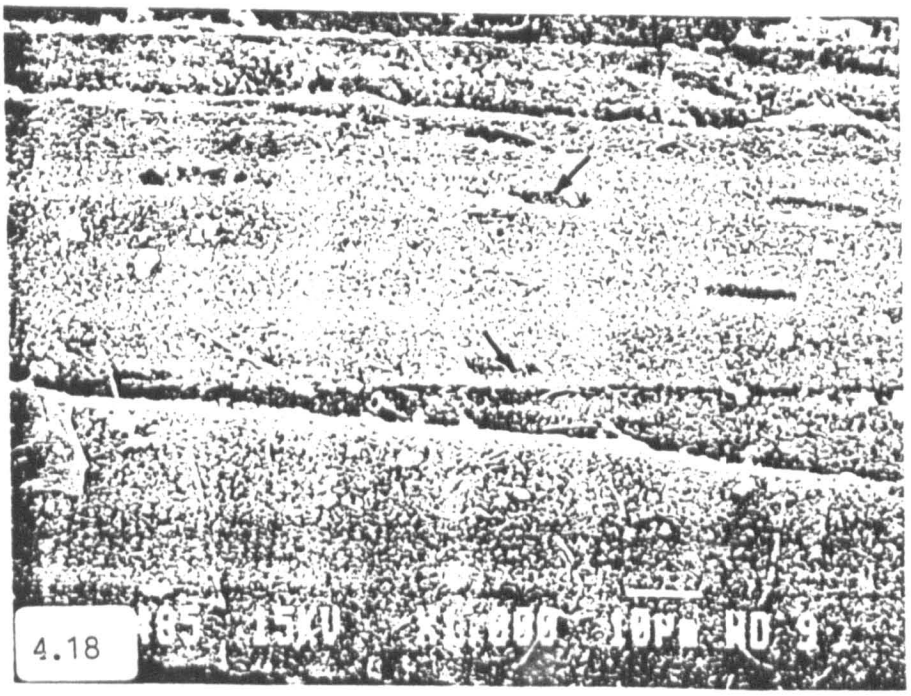
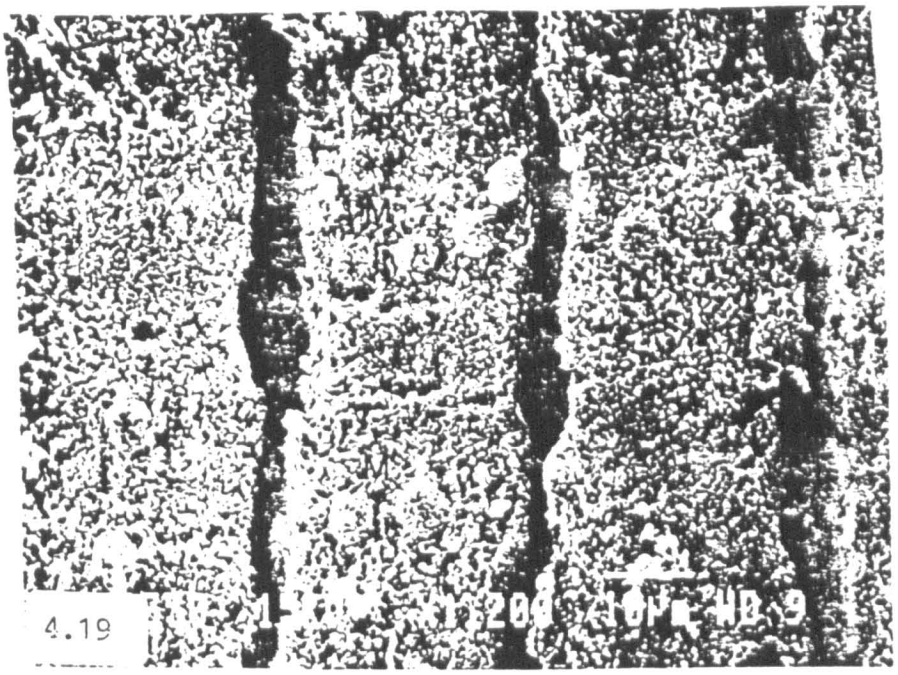

4.18

Figure 4.19: SEM. Three striated red muscle fibres replaced by microgranular apatite. Each fibre has a number of mitochondria ('M') on its external surface. The mitochondria are spherical (≈3μm diameter) and preserved as an internal mould by microgranular apatite. stub 96.

Figure 4.20: SEM. A mitochondrion on the external surface of a striated muscle fibre. The mitochondrion is preserved as both an external- ('E') and internal-mould ('I') by microgranular apatite. The un-mineralized zone represents the intermembrane space. The internal un-mineralized spaces may represent the position of cristae ('C').

The inter-membrane space is irregular and relatively wide, and the cristae are small, isolated, and reduced in numbers in comparison to pristine mitochondria. This implies this organelle to have experienced some decay. stub 81.

Figure 4.21: SEM. Two striated muscle fibres replaced by microgranular apatite. One of the fibres has mitochondria preserved on its external surface as un-mineralized 'scars' (arrowed) which represent their former point of attachment to the fibre. The mitochondria appear to have protected the underlying fibre from being coated with microgranular apatite but were themselves not phosphatized. Destroyed.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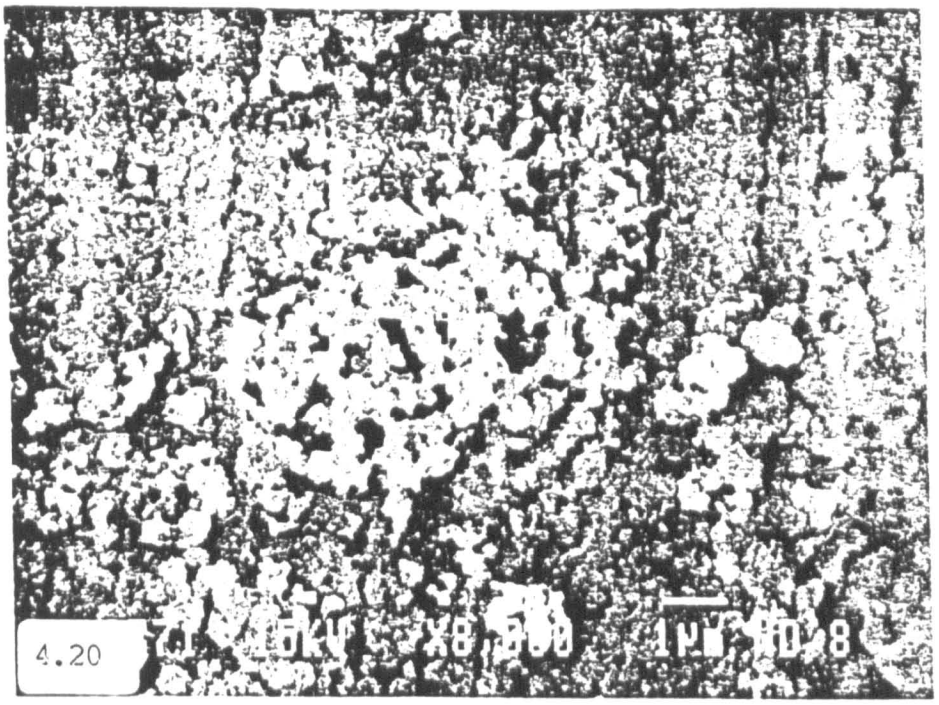
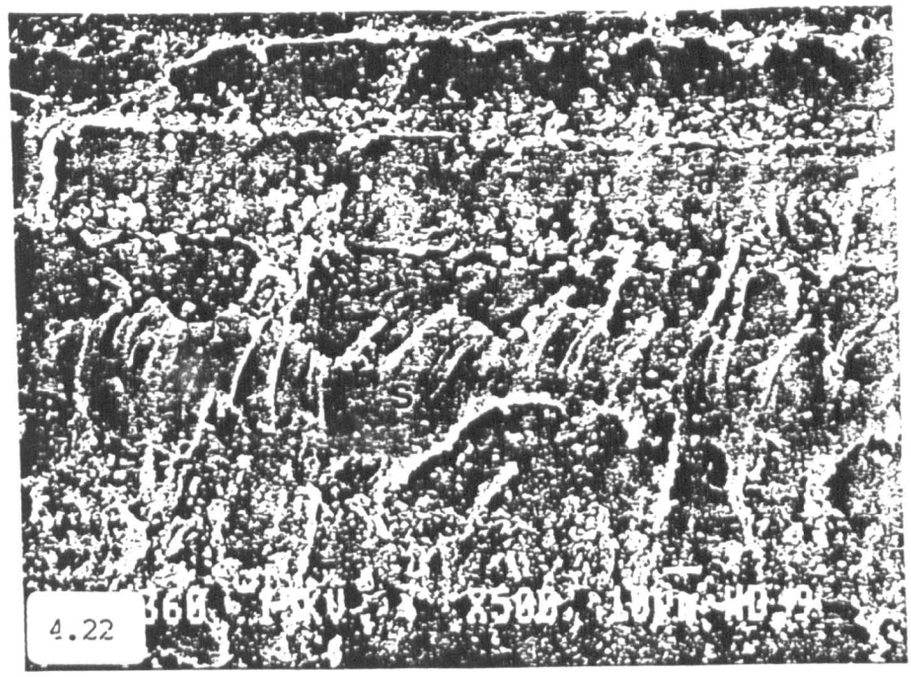

4.20

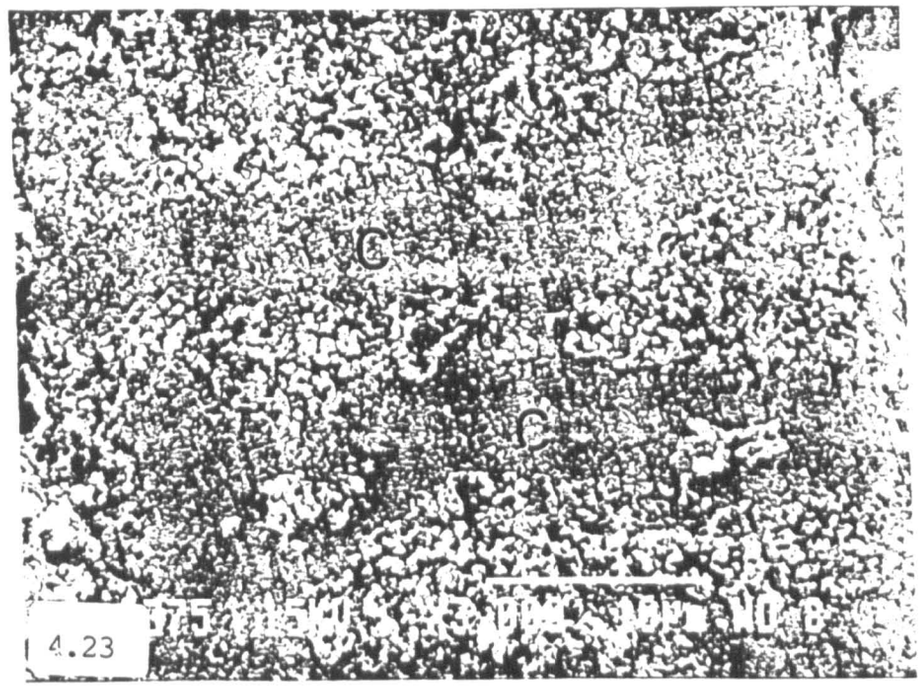

4.21

Figure 4.22: SEM. Striated muscle fibres replaced by microgranular apatite. The sarcolema ('S') of two of the fibres is partially preserved as an internal mould. Countersunk, transverse capillary loops ('C') are preserved under and slightly beyond the sarcolema. The former positions of the capillary loops on the surface of the rest of the fibres are defined by slight sinusoidal depressions ('D'). stub 54.

Figure 4.23: SEM. A striated muscle fibre with well preserved banding. The former positions of two transverse capillary loops ('C') are defined by sinusoidal areas on the fibre which have not been coated with microgranular apatite: the capillaries 'protected' these areas from mineralization but were themselves not mineralized. stub 53.

Figure 4.24: TEM. Well banded (running NE-SW) striated muscle fibre replaced by microgranular apatite. The T-tubules and sarcoplasmic reticulum ('T') are preserved as small (≈100nm diameter) un-mineralized hollows between laterally adjacent sarcomeres. Destroyed.


4.22


C
C
4.23

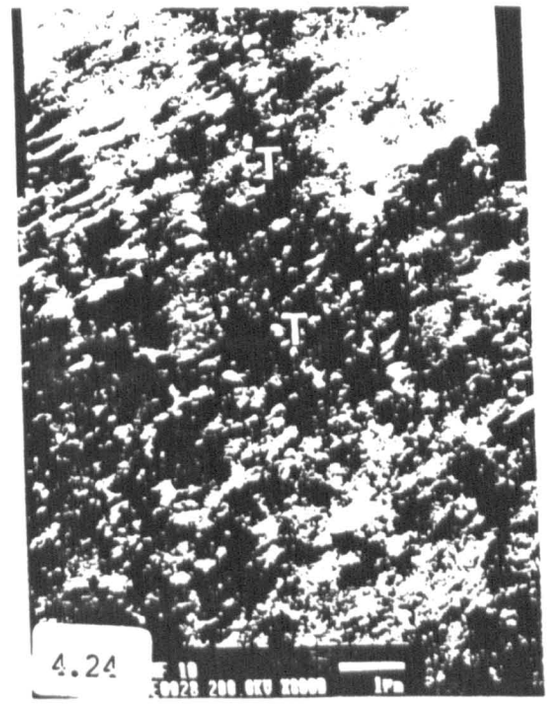
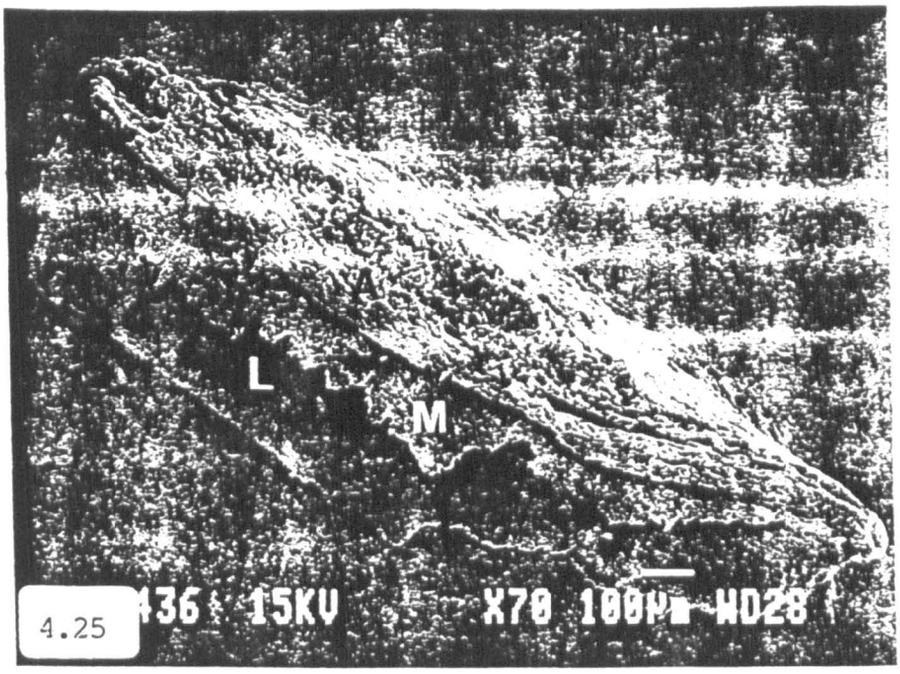
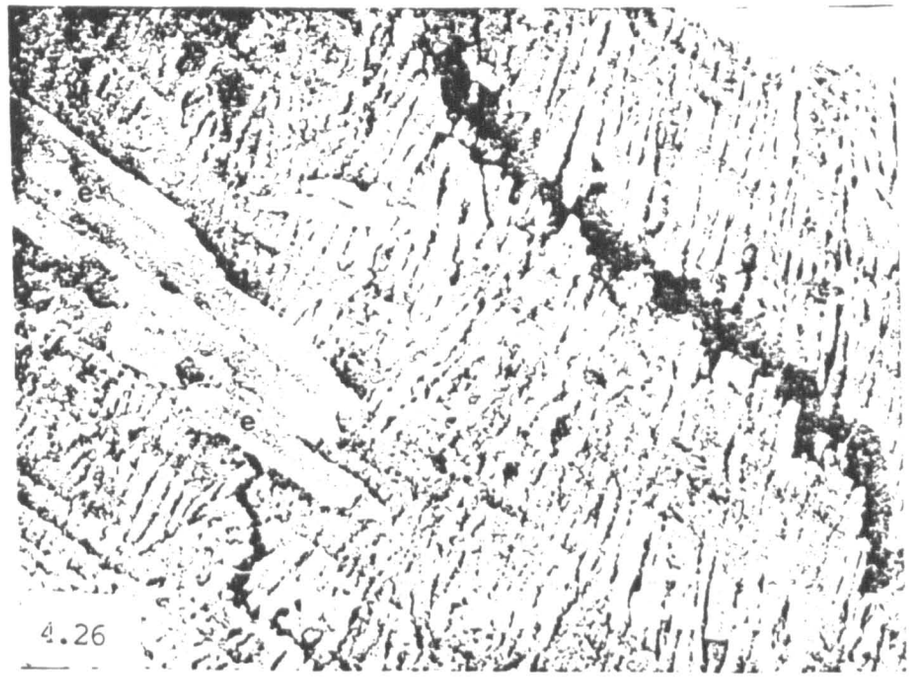

T
T
4.24

Figure 4.25: SEM. A section of an artery displaying two distinct layers surrounding the lumen ('L'). The tunica media ('M') consists of thin, circularly-arranged smooth muscle and elastic fibres each up to 100μm in length. The tunica adventitia ('A') consists of connective fibres running longitudinally along the vessel and is relatively thick (≈80μm). Both are replaced by microgranular apatite. stub 102.

Figure 4.26: SEM. The tunica media of an artery clearly preserving the circularly-arranged smooth muscle and elastic fibres. On these, and in direct contact with the lumen, are a few internal moulds of simple squamous epithelial cells ('E'). stub 102.

Figure 4.27: SEM. The tunica media ('M') and tunica adventitia ('A') of an artery. The tunica media consists of circularly-arranged smooth muscle and elastic fibres. The tunica adventitia consists of a thick band (≈80μm) of longitudinally-arranged connective fibres. Both are replaced by microgranular apatite. stub 102.


L
M
4.25
436 15KV X70 100µm WD28


e
e
4.26

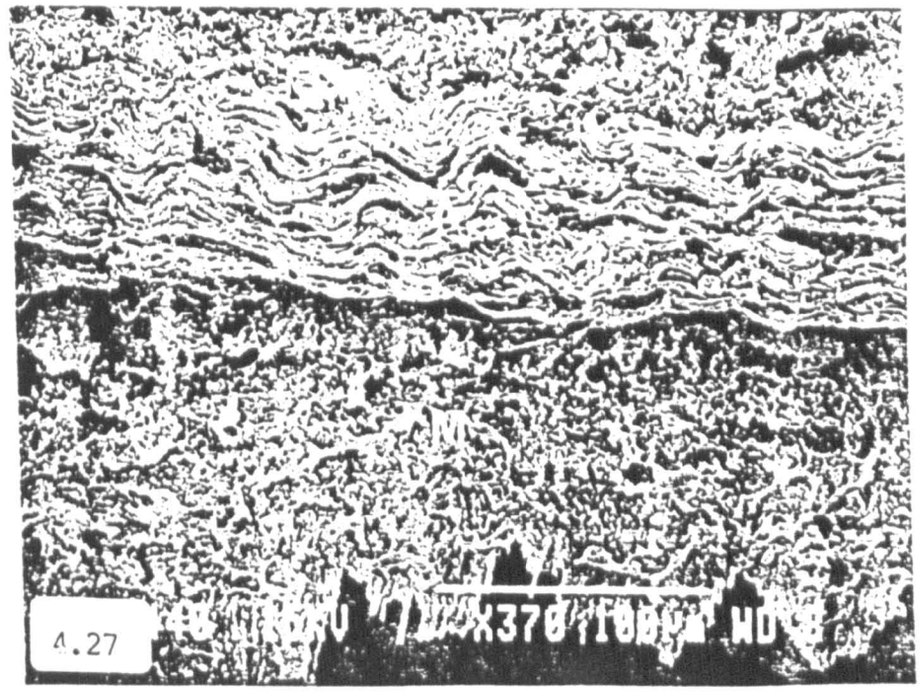
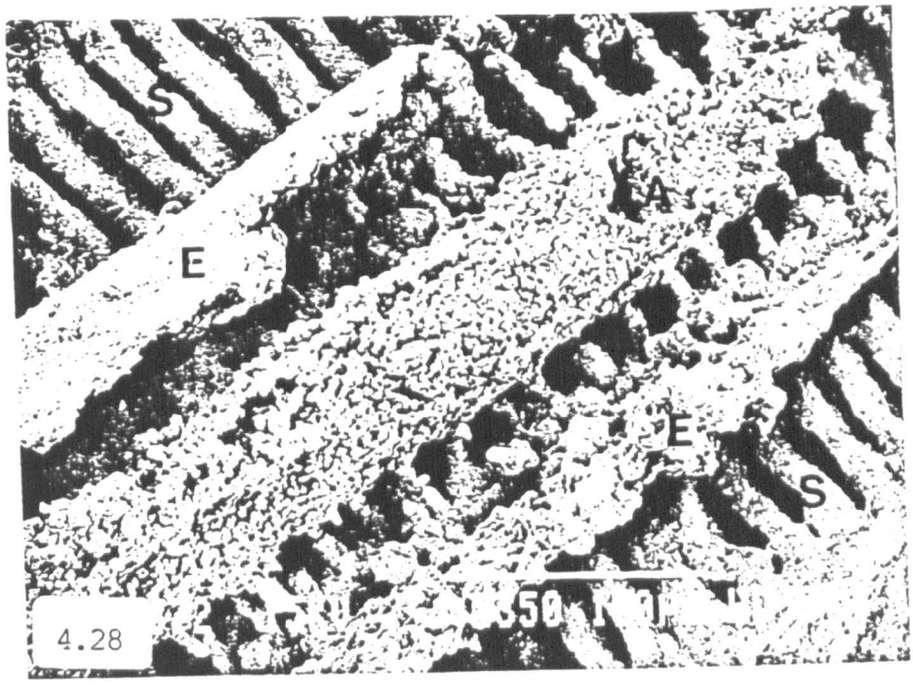

4.27

Figure 4.28: SEM. Efferent artery of the gill apparatus of *Notelops* sp. The artery ('A'), secondary gill lamellae ('S'), and protective epithelium ('E') surrounding the efferent artery are all pseudomorphed by morphotype 1a microbes. The ultrastructure of the artery is not preserved. stub 86.

Figure 4.29: SEM. Efferent artery of the gill apparatus of *Rhacolepis* sp. The entire gill apparatus is pseudomorphed by morphotype 1a microbes. The longitudinal connective fibres of the artery ('A') are well preserved, and ?epithelial cells ('E') are preserved on the external surface of its surrounding thick epithelium. The secondary gill lamellae of adjacent gill filaments are fused to one another by a thin layer of epithelium ('Z'). Removal of the epithelium demonstrates the fusion to be only superficial: the secondary gill lamellae of adjacent filaments only abut against one another (arrowed). stub destroyed.


S
E
A
E
S
4.28

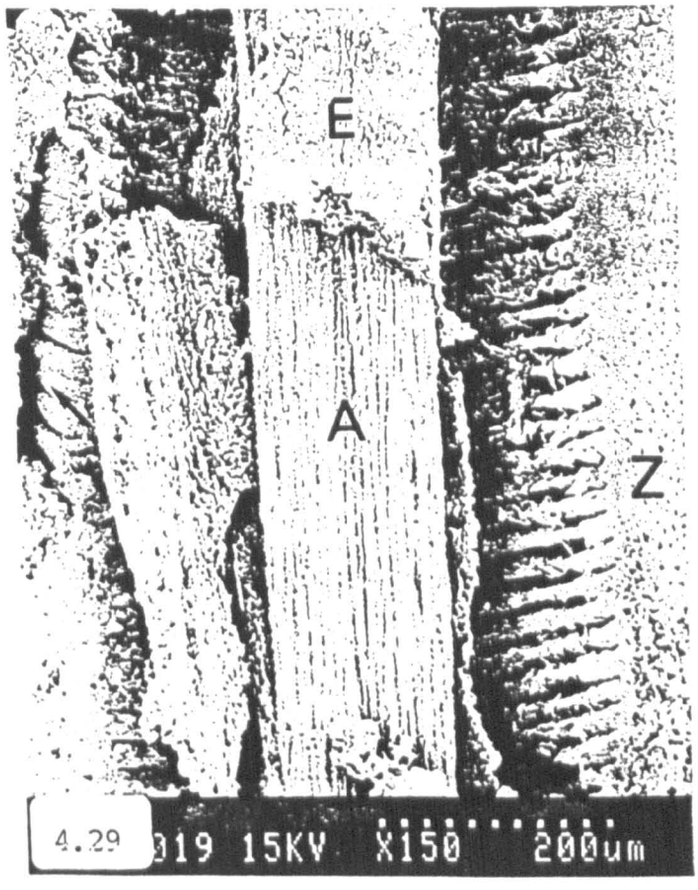
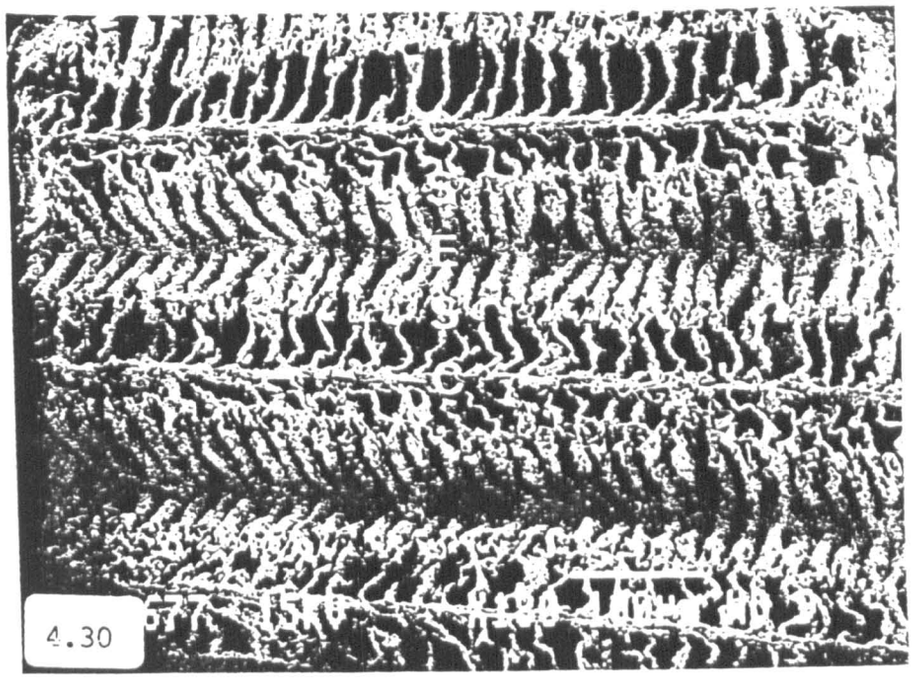

E
A
Z
4.29
019 15KV X150 200um

Figure 4.30: SEM. Usually, the gill apparatus of *Rhacolepis* sp. and *Notelops* sp. breaks along a plane normal to the distal tip of each gill ray. This figure gives the proximal view of the distal fused part of the gill apparatus. The gill rays would be positioned normal to the photomicrograph directly below the central sinus' ('C'). The distal ends of the secondary gill lamellae ('S') are well preserved and are fused by the connective epithelial tissue ('E'). stub 86.

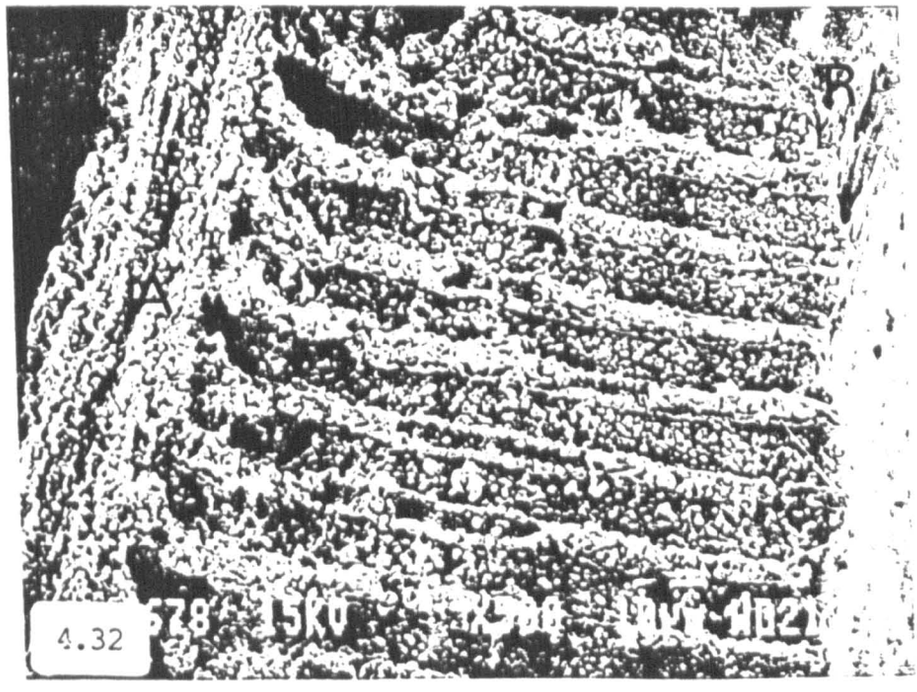
Figure 4.31: TEM. An individual secondary gill lamella of a *Notelops* sp. coated by morphotype 1a microbes. The central sinus ('C') is preserved as an internal mould by microgranular apatite. The sinusoid ('S') remains largely un-mineralized. Micro E9.

Figure 4.32: SEM. A gill ray of a *Tharrias* sp. The epithelial tissue ('E') coating the gill ray ('R'), the tubular secondary lamellae ('S'), and the afferent artery ('A') are replaced by morphotype 1a microbes. The secondary gill lamellae are preserved as external moulds. stub 64.



4.30

4.31

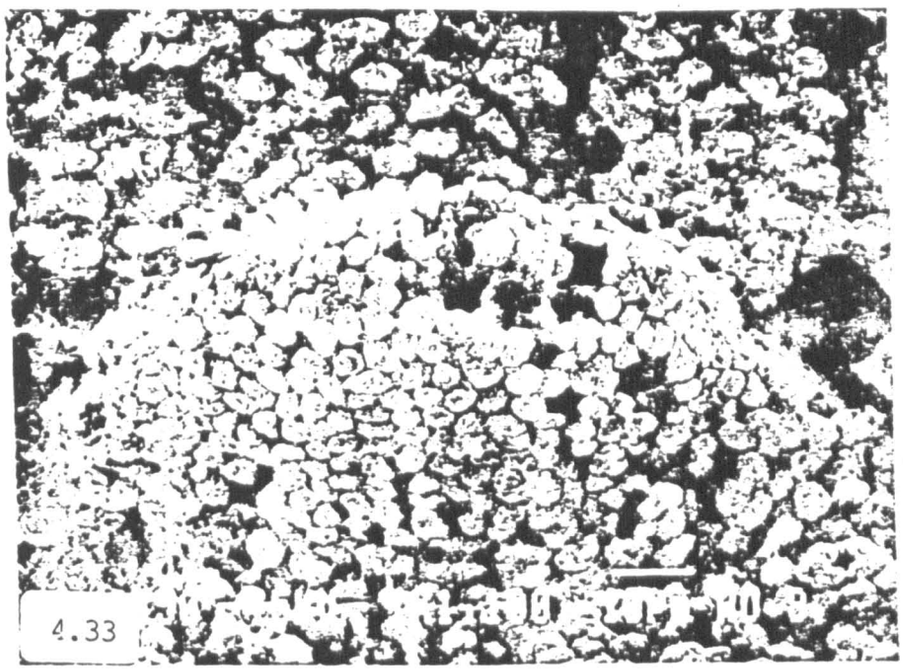


4.32

Figure 4.33: SEM. A primary fold on the rectum of a *Notelops* sp. The epithelium is covered with epithelial cells preserved as internal moulds by microgranular apatite. The underlying squamous epithelium ('S') is only patchily phosphatized. stub 101.

Figure 4.34: SEM. A longitudinal primary fold in the alimentary tract of a *Rhacolepis* sp. Removal of the epithelial cells ('E') reveals the underlying squamous epithelium ('S') to be replaced by a combination of microbes and microgranular apatite. The squames are rather more elongate and flatter than the epithelial cells. The underlying stratum compactum ('C') is densely mineralized and replaced by microgranular apatite. stub 28.

Figure 4.35: SEM. Epithelial cells of the alimentary tract of a *Notelops* sp. preserved as hollow internal moulds by microgranular apatite. The plasma membranes ('P') are preserved as thin un-mineralized gaps between each cell. Microvilli ('M') are preserved as internal moulds on some of the cells. stub 135.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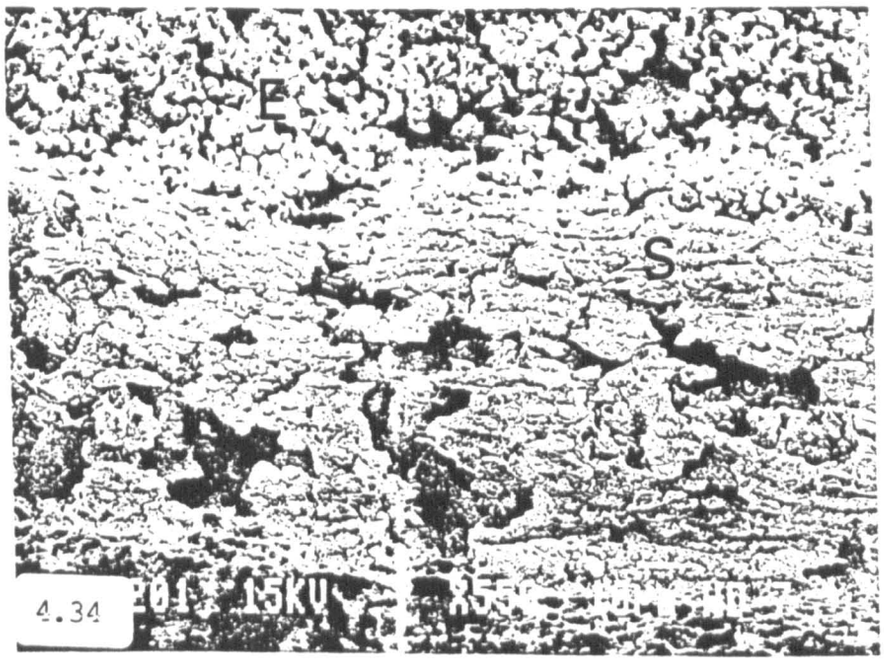

E
S
4.34

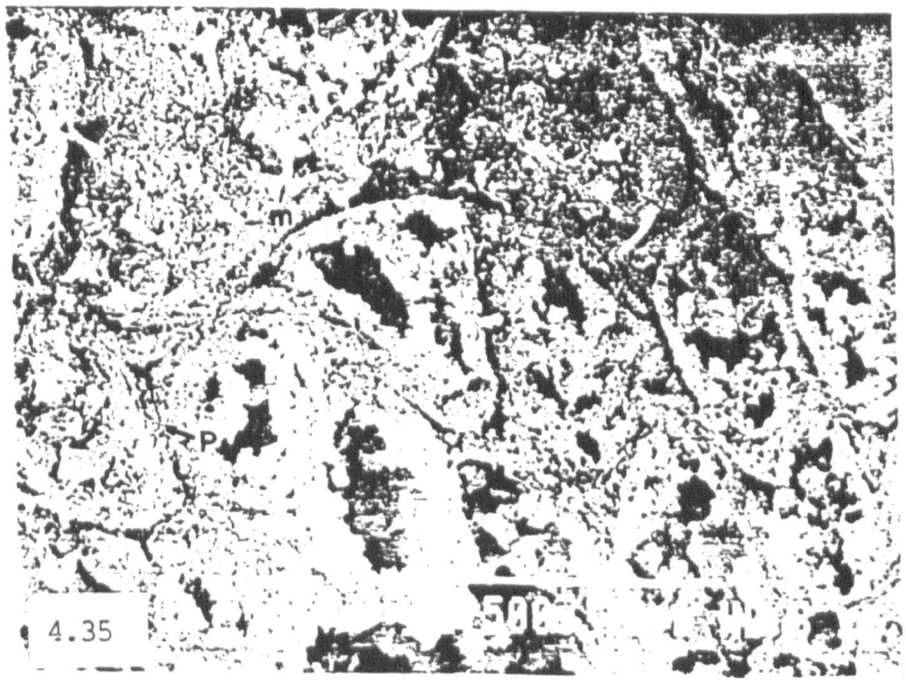
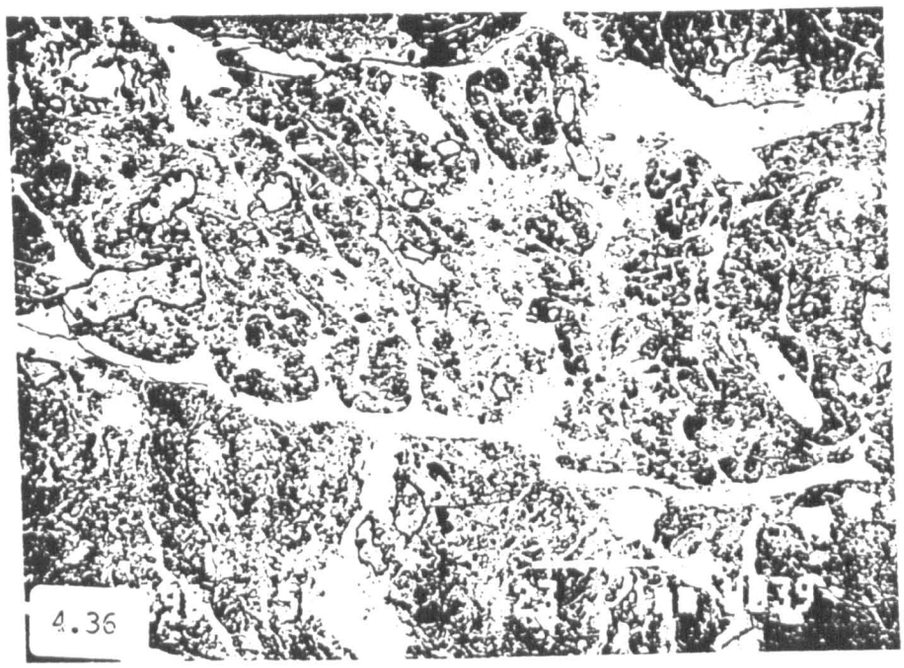

m
P
4.35

Figure 4.36: SEM (backscatter image of thin section). Two primary folds of a phosphatized spiral coprolite separated by calcite (presumably occupying the former position of the mucous membrane). One of the primary folds is refolded into secondary folds. The coprolitic material is completely homogenized suggesting it to have been fully digested. Slide A.

Figure 4.37: SEM. An oval gastric residue composed of disarticulated fragments of shrimps cemented by amorphous coprolitic material. The carapaces of the shrimps display evidence of etching (arrowed) by gastric fluids. stub 66.

Figure 4.38: SEM (EDAX elemental maps). Phosphorus ('P') and calcium ('Ca') elemental maps, and backscattered image of the ovaries and wall of the alimentary tract ('W') of a gravid female *Notelops* sp. The eggs are preserved as external moulds and are at various stages of 'maturity'. One egg (arrowed) displays some signs of internal structure. Primary longitudinal folds ('F') are preserved in the alimentary tract. Slide B.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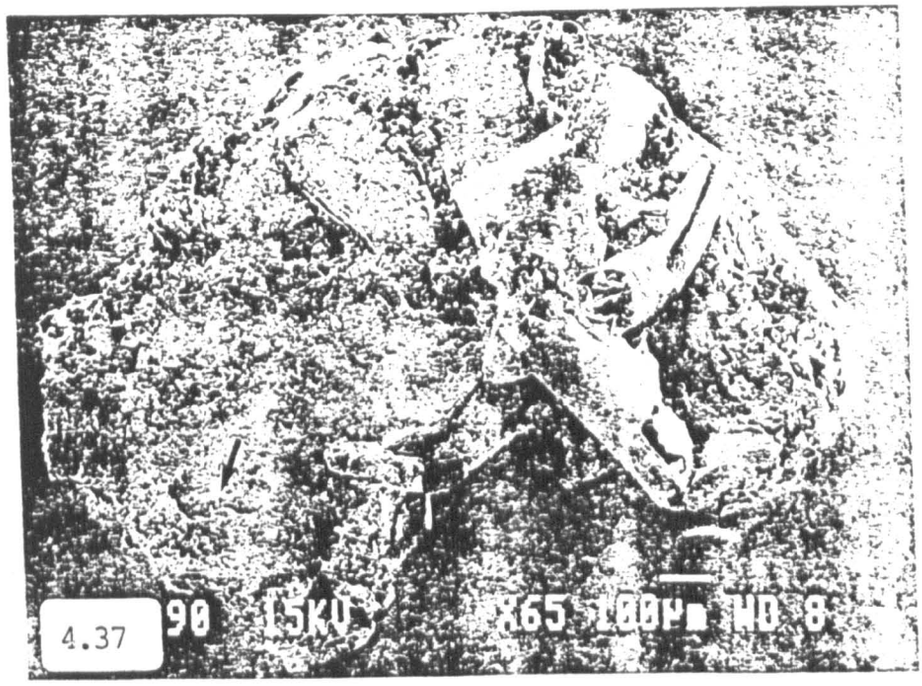
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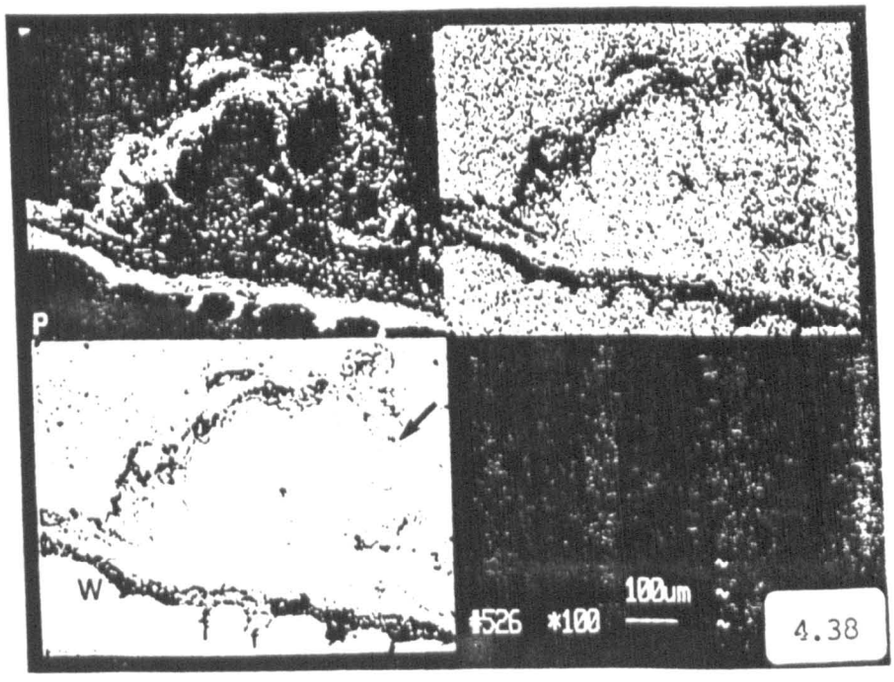
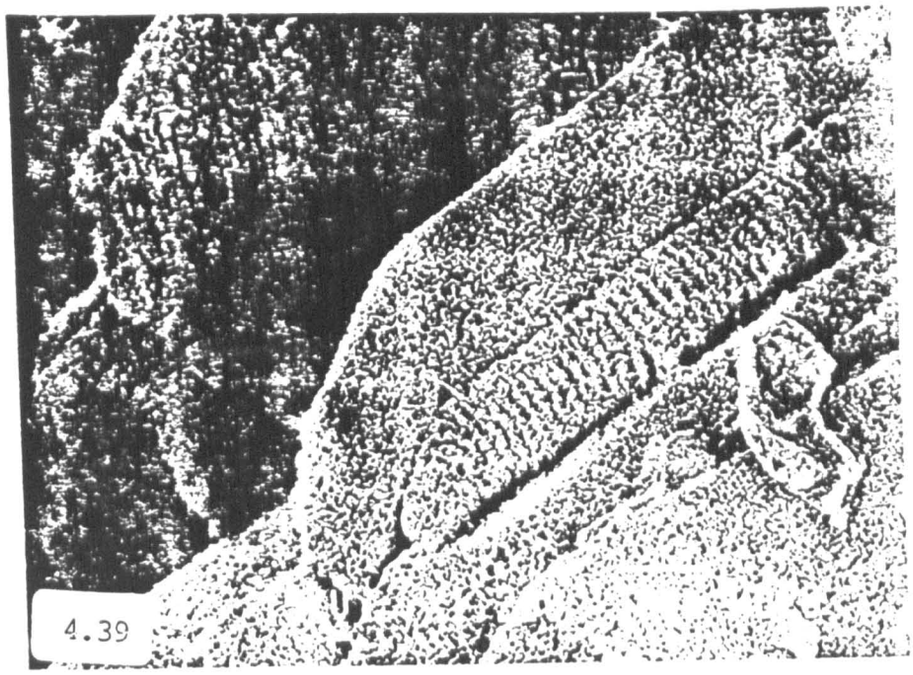

P
W
100um
4.38

Figure 4.39: SEM. Striated muscle fibres from the wing membrane of a pterosaur. The muscle fibres are replaced by microgranular apatite and consist of a series of stacked discs separated by un-mineralized M-lines. stub is part of DNPM D6M 488 LE.

Figure 4.40: SEM. Phosphatized planktonic larval stage of a bivalve from the stomach of a *Rhacolepis* sp. The valves display a complex history of dissolution, apatite deposition, and microbial infestation. This has resulted in a complex arrangement of internal- and external-moulds, and replacements. 3cm=100μm. stub 133.


4.39

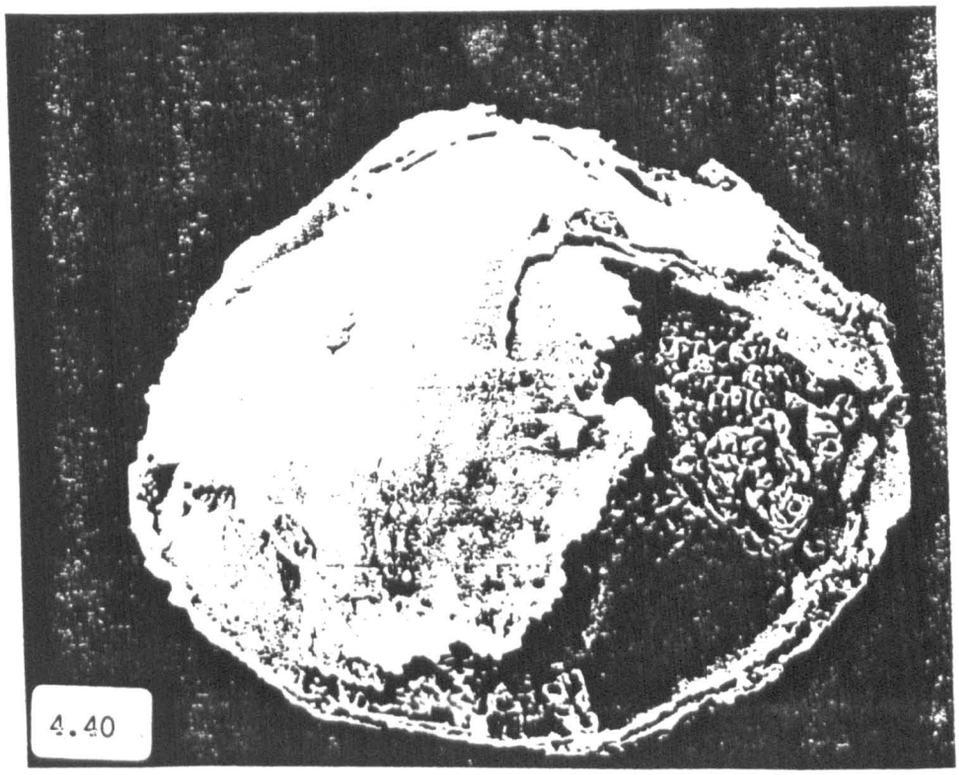
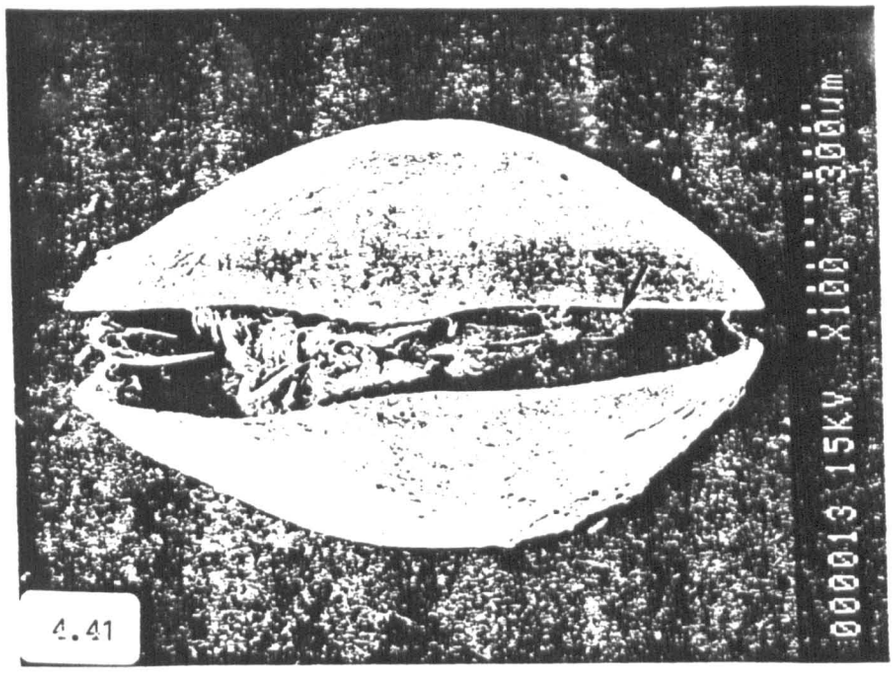

4.40

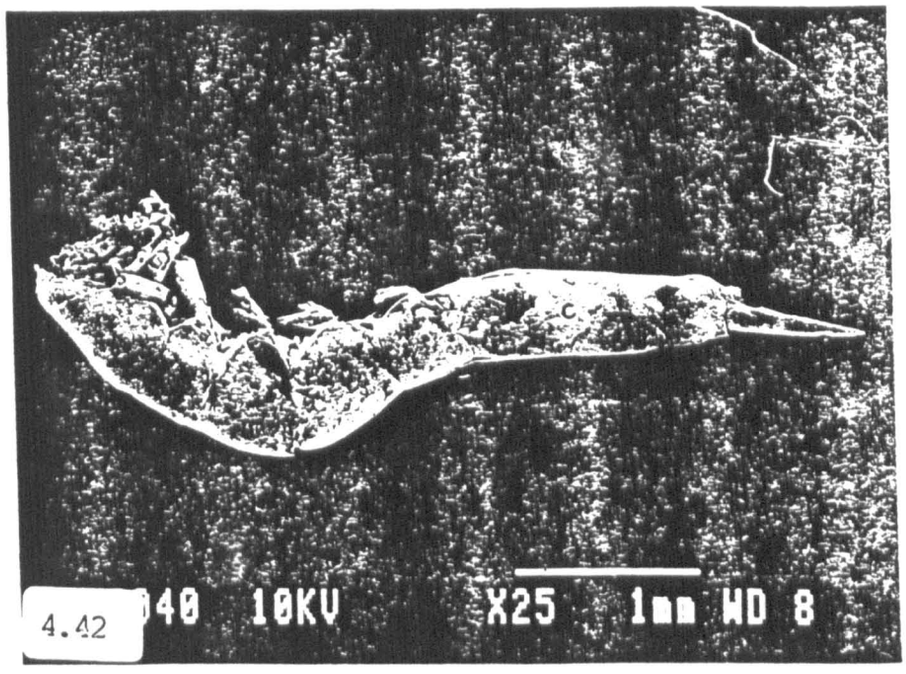
Figure 4.41: SEM. Partially open phosphatized ostracode (Pattersoncypris sp. with appendages. Even bristles on the appendages are preserved. The body and appendages are replaced by microgranular apatite, whilst the carapace displays at least one coating of apatite, and the space between the carapace and body is infested by microbes (arrowed). stub not accesioned, held by D.M. Martill at Leicester University.

Figure 4.42: SEM. Well preserved abdominal and caudal region of a decapod shrimp (undet. sp.) from the stomach of a *Rhacolepis* sp. The carapace, distal portion of the appendages, and uropods were presumably disarticulated during ingestion. The exoskeleton is both replaced ('R') and coated ('C') by apatite. stub LEIUG 107850.

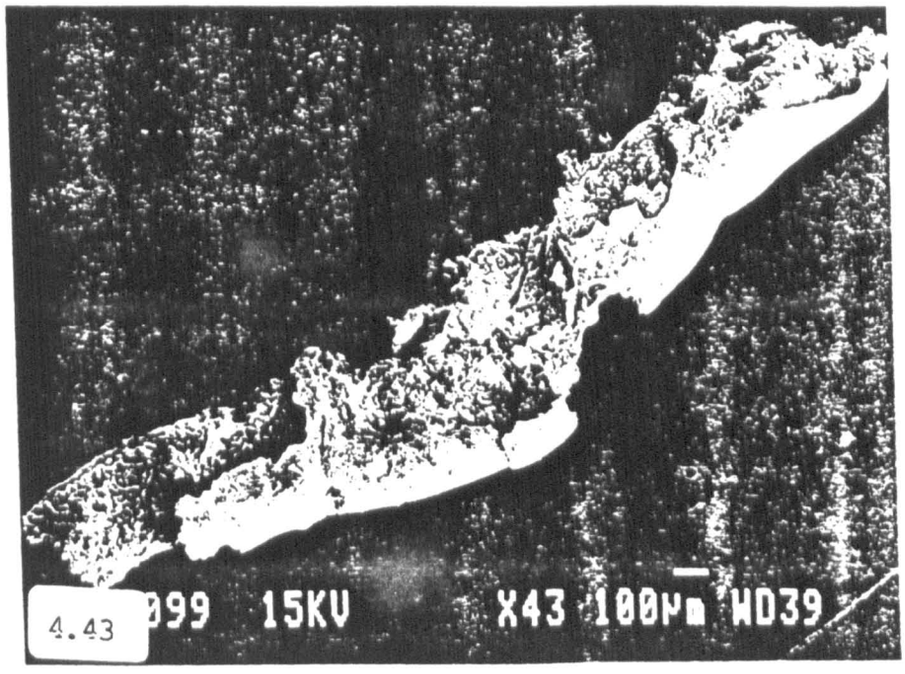
Figure 4.43: SEM. Poorly preserved abdominal and caudal region of a decapod shrimp (undet. sp.) from the stomach of a *Rhacolepis* sp. The appendages, telson, and carapace are absent, and much of the exoskeleton is damaged or lost. The musclature however is well preserved. stub 7


000013 15KV X100 300µm

4.41


40 10KV X25 1mm WD 8

4.42


099 15KV X43 100µm WD39

4.43

Figure 4.44: SEM. Exceptionally well preserved compound eye of a decapod shrimp (undet. sp.) from the stomach of a *Rhacolepis* sp. Each lens is replaced by microgranular apatite with remarkable precision. stub LEIUG 107848.

Figure 4.45: SEM. Three exopods of a decapod shrimp (undet. sp.) from the stomach of a *Rhacolepis* sp. Linear rows of setae are preserved by a combination of internal- and external-coatings of microgranular apatite. stub LEIUG 107849.


4.44
000 20KV X150 200um

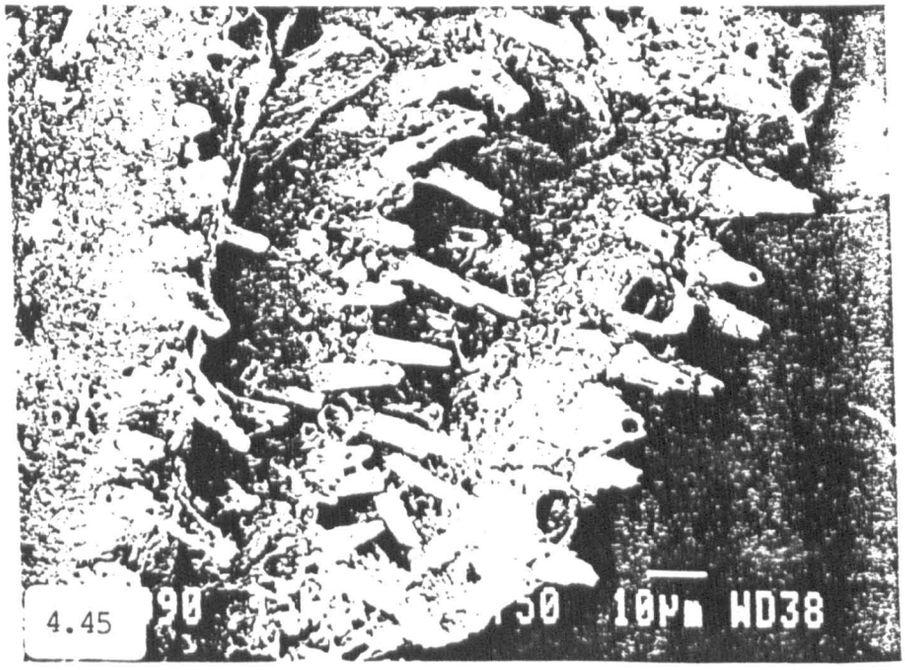
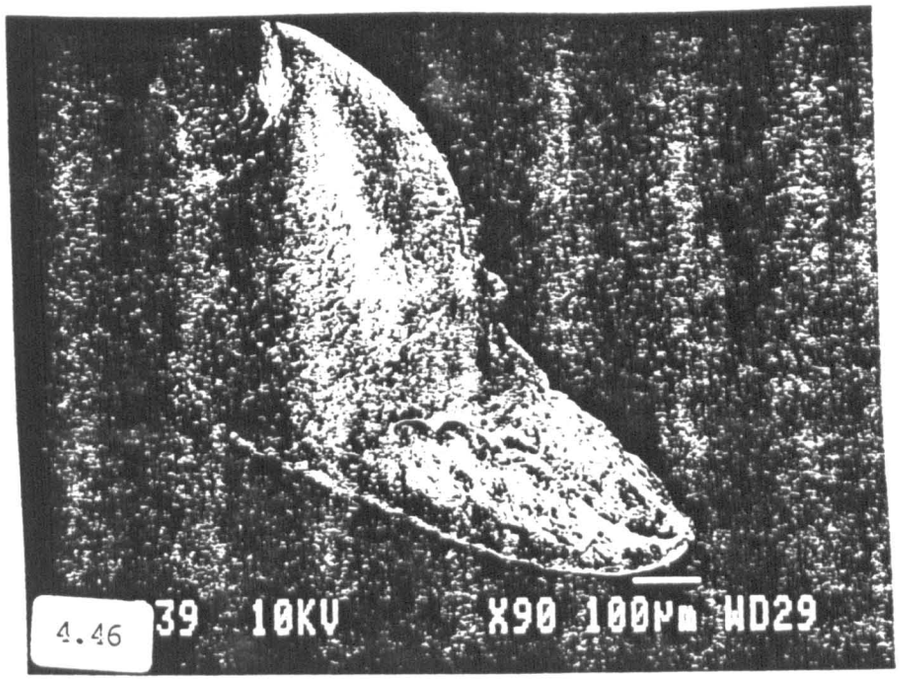
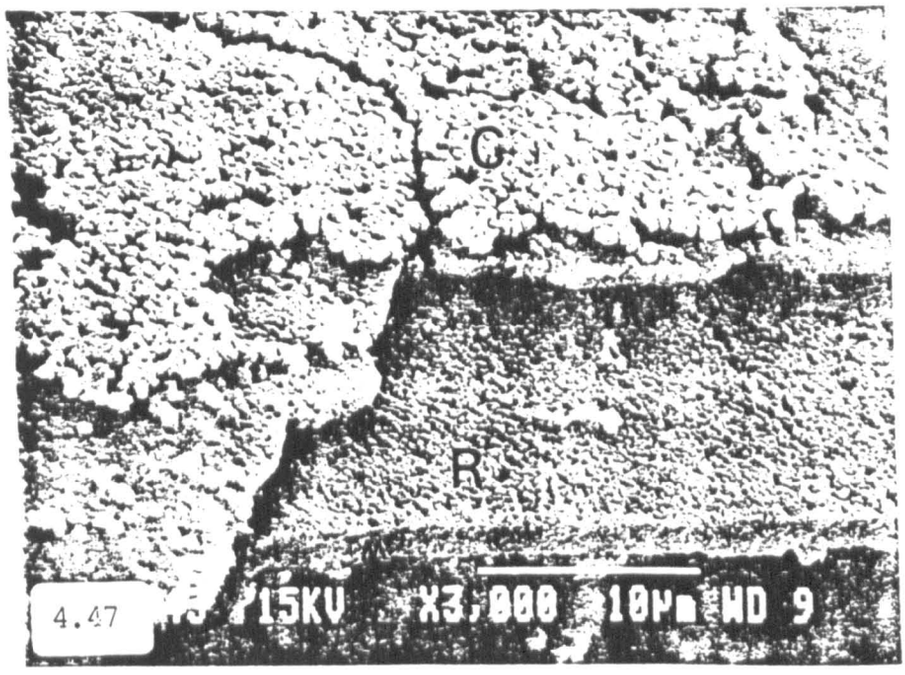

4.45
10µm WD38

Figure 4.46: SEM. Chelicera of a decapod shrimp (undet. sp.) from the stomach of a *Rhacolepis* sp. stub 2.

Figure 4.47: SEM. The exoskeleton of a decapod shrimp (undet. sp.) from the stomach of a *Rhacolepis* sp. preserved by a combination of microbial coatings ('M'), inorganic coatings ('C'), and inorganic replacements ('R'). Frequently, one or more of the layers of the exoskeleton have experienced dissolution ('D'). stub 14.

Figure 4.48: SEM. Internal mould of an appendage of a decapod shrimp (undet. sp.) from the stomach of a *Rhacolepis* sp. The internal mould is composed of inorganic microspheres. The occurrence of disorganised microspheres towards the end of the appendage ('D') suggests mineralization to have accompanied the dissolution of the exoskeleton. stub 3.


4.46
39 10KV X90 100µm WD29


C
R
4.47
15KV X3,000 10µm WD 9

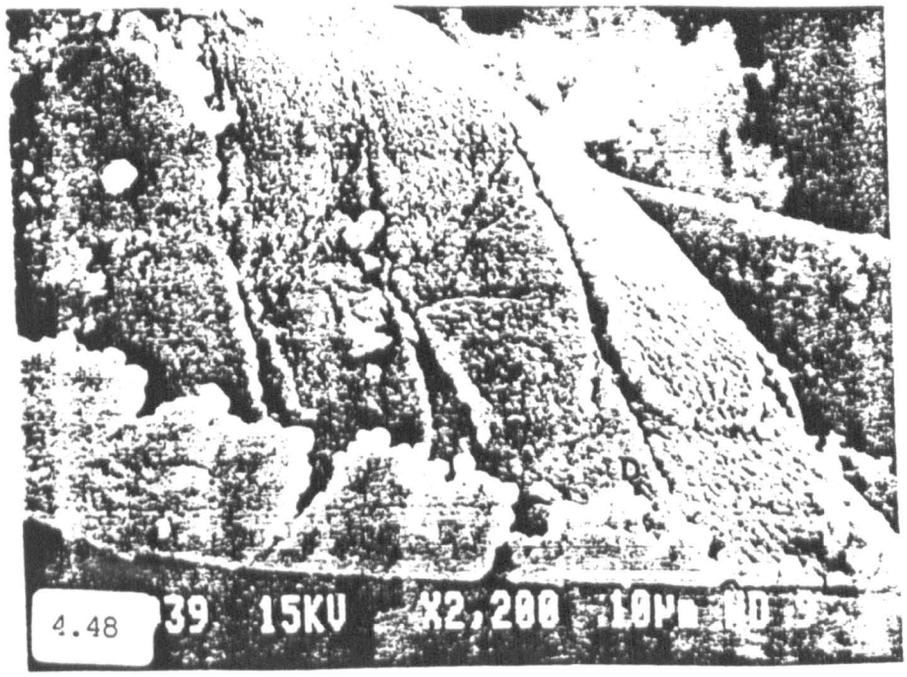
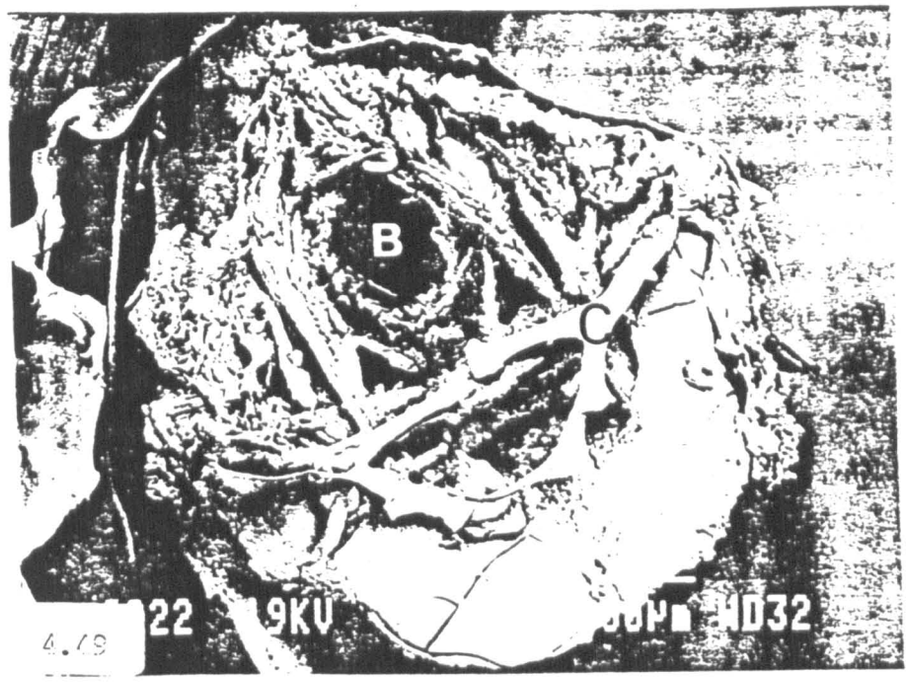
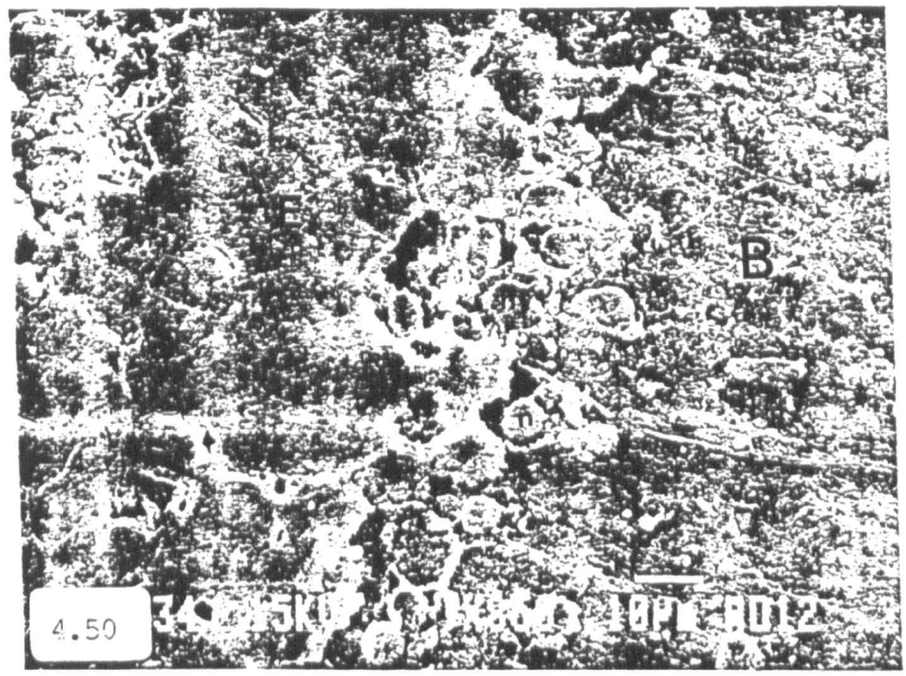

4.48
39 15KV X2,200 10µm

Figure 4.49: SEM. Exceptionally well preserved musclature at the abdomen/carapace junction of a decapod shrimp (undet. sp.) from the stomach of a *Rhacolepis* sp. The sternum ('C') is well preserved, but the intestine ('B') is un-mineralized. stub LEIUG 107849.

Figure 4.50: SEM. The basal membrane ('B') and sub-cuticular epithelial cells ('E') of many of the decapod shrimps (undet. sp.) from the stomachs of *Rhacolepis* sp. are well preserved. The epithelial cells and central cell nuclei ('N') are preserved as internal moulds by microgranular apatite. The basal membrane is replaced by microgranular apatite. stub 12.

Figure 4.51: SEM. Removal of the plasma membrane ('P') of the sub-cuticular epithelial cells of decapod shrimps (undet. sp.) from the stomachs of a *Rhacolepis* sp. reveals exceptionally well preserved internal moulds of cell nuclei ('N'). stub 12.


B
C
4.49


B
4.50

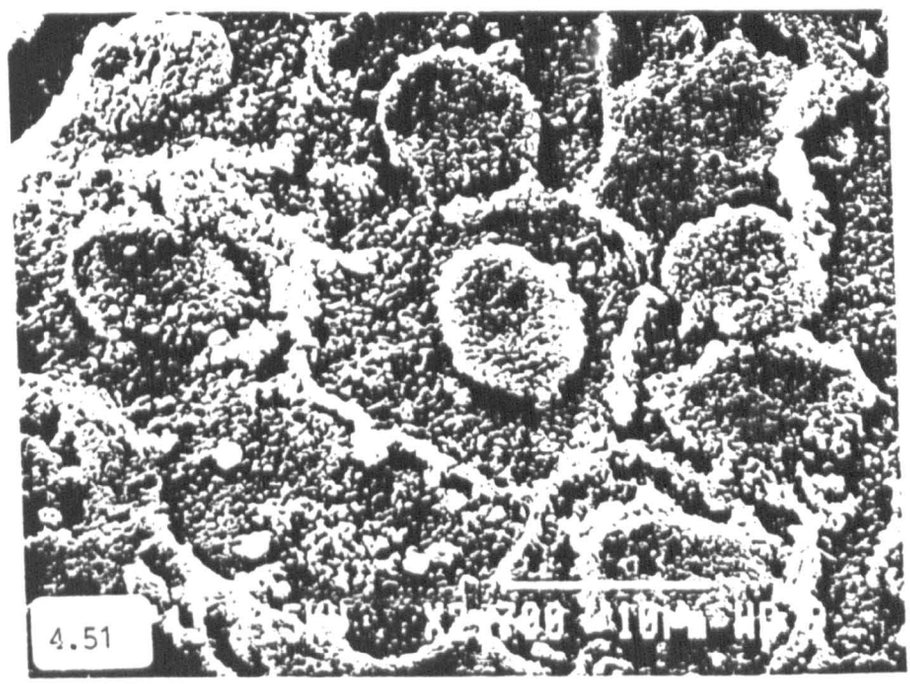
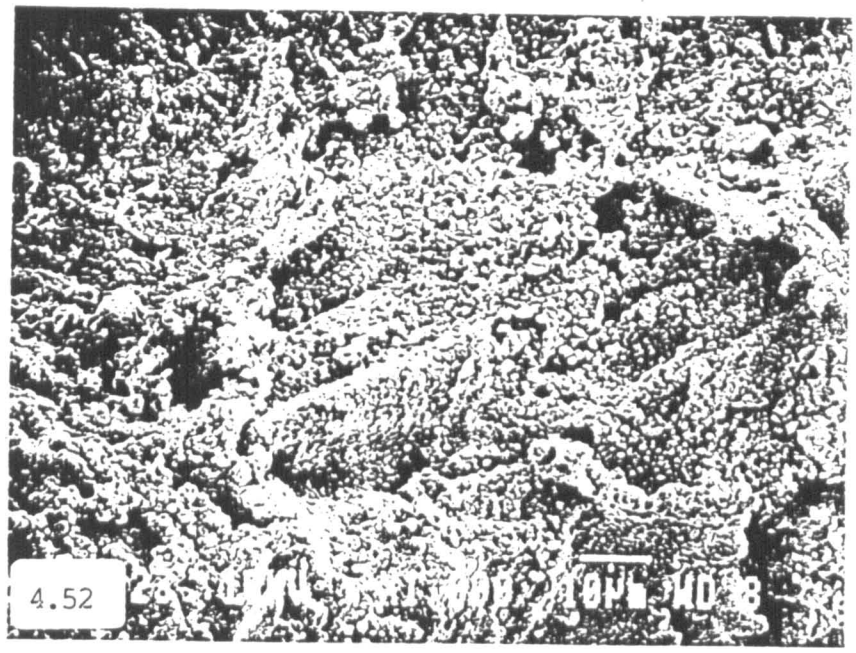

4.51

Figure 4.52: SEM. Sub-cuticular basal membrane of a decapod shrimp (undet. spec.) from the stomach of a *Rhacolepis* sp. The membrane is replaced by inorganic microspheres. stub 12.

Figure 5.1: SEM. Striated muscle of *Scombroclupea macrophthalma* (BMNH P4747) from the Haqel Basin. The muscle is entirely structureless and replaced by aggregates of inorganic microcrystalline apatite. stub destroyed.

Figure 5.2: SEM. Skeletal muscle fibres (running E-W) in a *Dastilbe* sp. from the Nova Olinda Member, Crato Formation. The fibres are preserved as hollow external moulds by ?microbes. stub120.


4.52


5.1

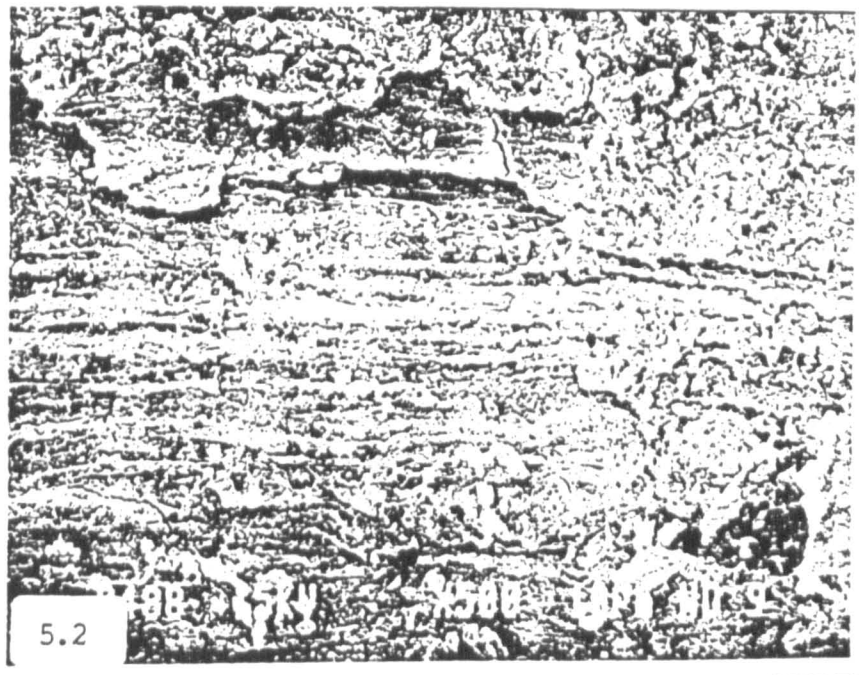
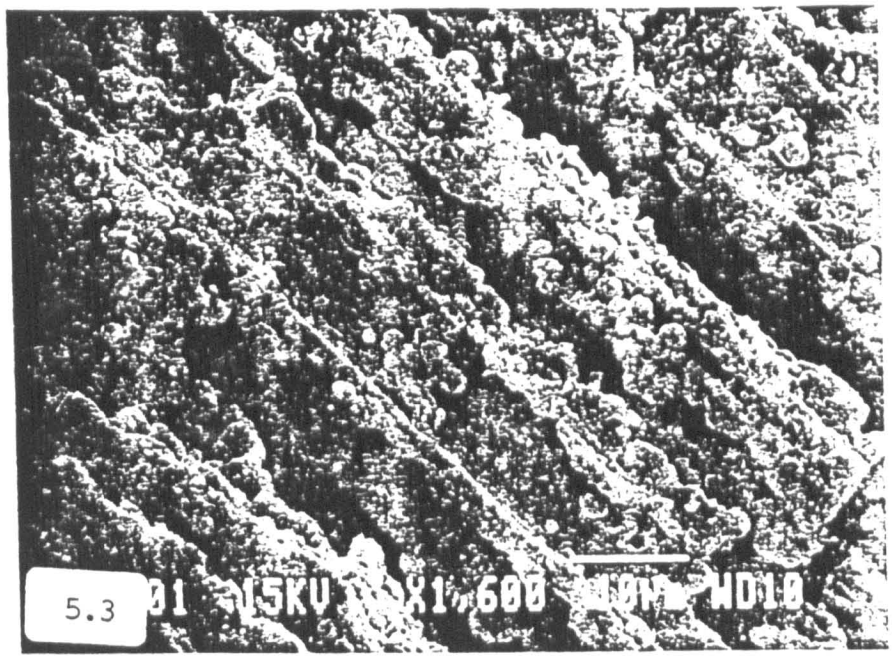

5.2

Figure 5.3: SEM. Muscle fibres (running NW-SE) from the mantle of *Laevitrigonia gibbosa* from the Portland Roach. The fibres are preserved as hollow external moulds by microbes. stub 117.

Figure 5.4: SEM. Folds in the alimentary tract of *L. gibbosa* from the Portland Roach. The tissue is both pseudomorphed and coated by microbes. stub 117.

Figure 5.5: SEM. Myofibrils (running NW-SE) preserved in muscle fibres from the mantle of *L. gibbosa* from the Portland Roach. The myofibrils are pseudomorphed with some precision by morphotype 1b microbes. The position of the microbes is dictated by the ultrastructure of the muscle fibre. stub 118.


5.3


5.4

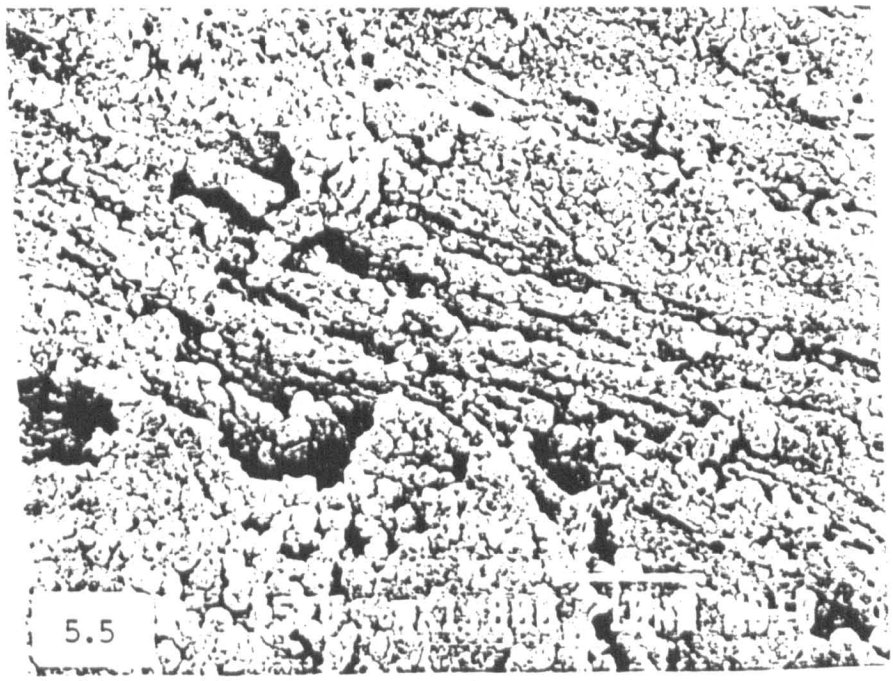
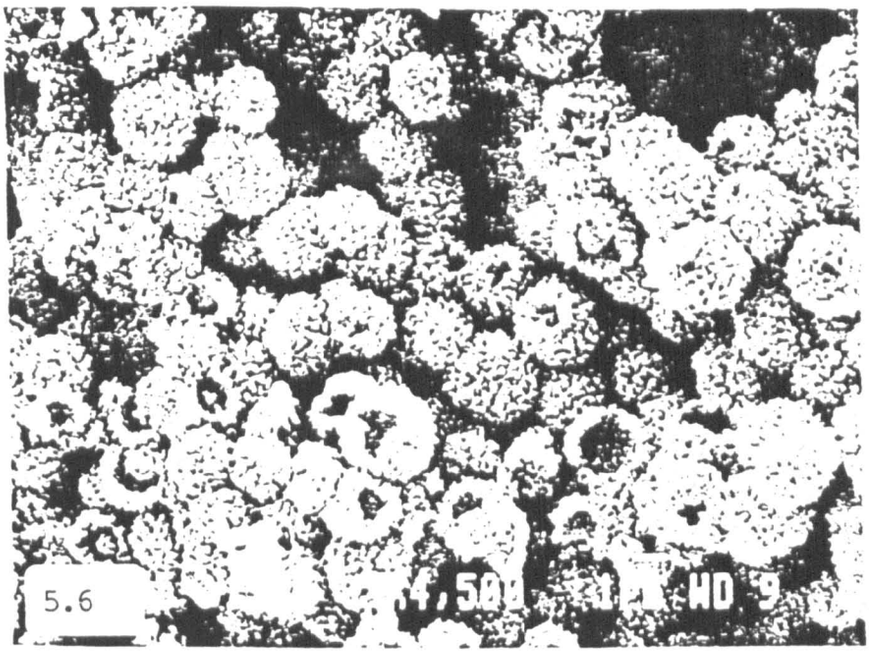
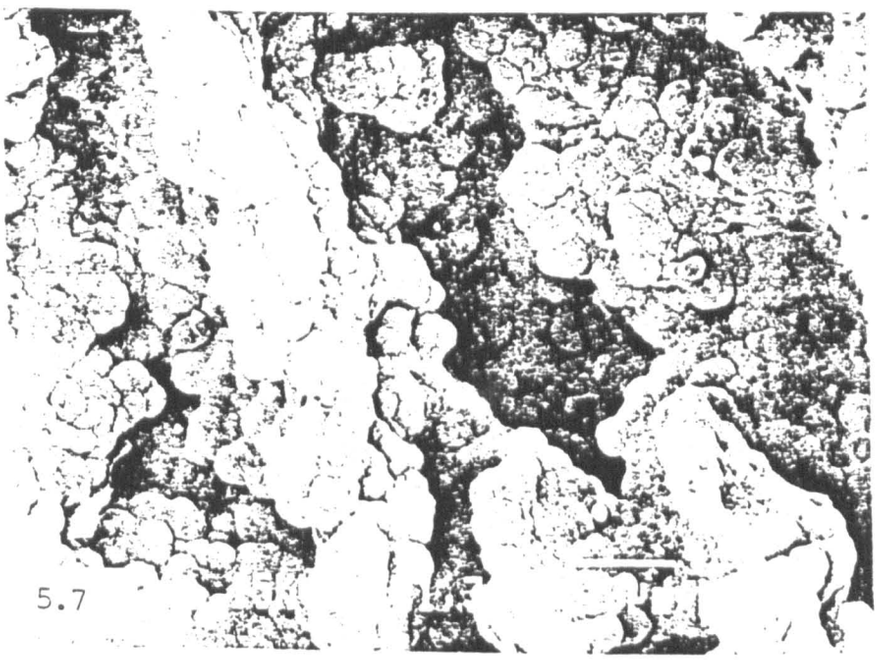

5.5

Figure 5.6: SEM. Morphotype 4 microbes pseudomorphing the alimentary tract of a *L. gibbosa* from the Portland Roach. The distinctive framboidal morphology of this morphotype is well developed. Each microbe is hollow and contains an organelle preserved as an external mould. stub 116.

Figure 5.7: SEM. Morphotype 2 microbes coating the gills of a *L. gibbosa* from the Portland Roach. The tissue's structure is only poorly reproduced by the microbes. stub 148.

Figure 5.8: SEM. Morphotype 1b microbes coating the external surface of ooliths caught in the mantle cavity of a *L. gibbosa* from the Portland Roach. The characteristic 'cauliflower-like' external surface of this morphotype is well developed, and the microbes are incised by thin cylindrical depressions identical to those preserved in morphotype 1b microbes from the Romualdo Member. stub 158.


5.6

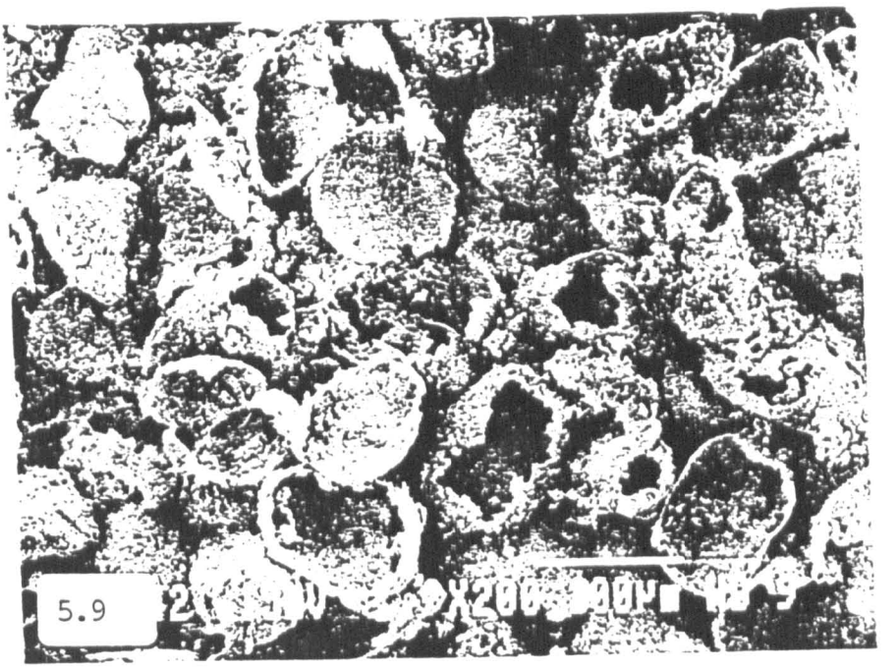

5.7


5.8

Figure 5.9: SEM. Phosphatized ooliths in the mantle cavity of a *L. gibbosa* from the Portland Roach. The ooliths are preserved as external moulds by microbes. stub 158.

Figure 5.10: SEM. Morphotype 4 microbes pseudomorphing the tentacles of *Plesioteuthis prisca* from the Solnhofen Limestone (BMNH C46871BMNH). None of the muscle's original structure is preserved. Contrary to morphotype 4 microbes from the Romualdo Member, these microbes impinge on one another to a greater extent, and their organelles are not preserved. stub 91.

Figure 5.11: SEM. Muscle fibres (running NW-SE) from the tentacles of *Plesioteuthis prisca* from the Solnhofen Limestone (BMNH C46871). The fibres are crudely pseudomorphed by microbes (?morphotype 4). stub 91.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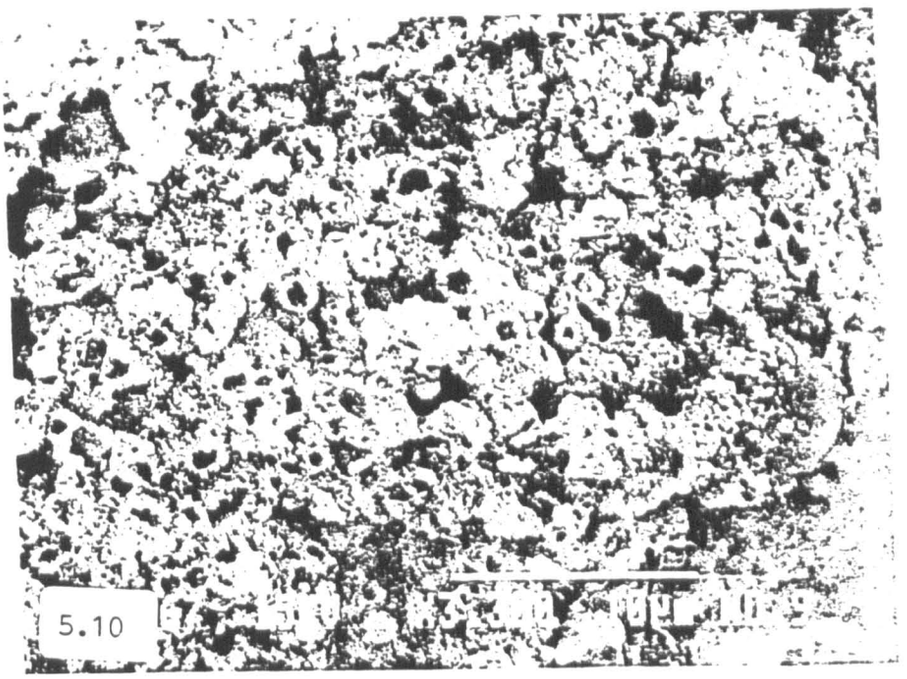
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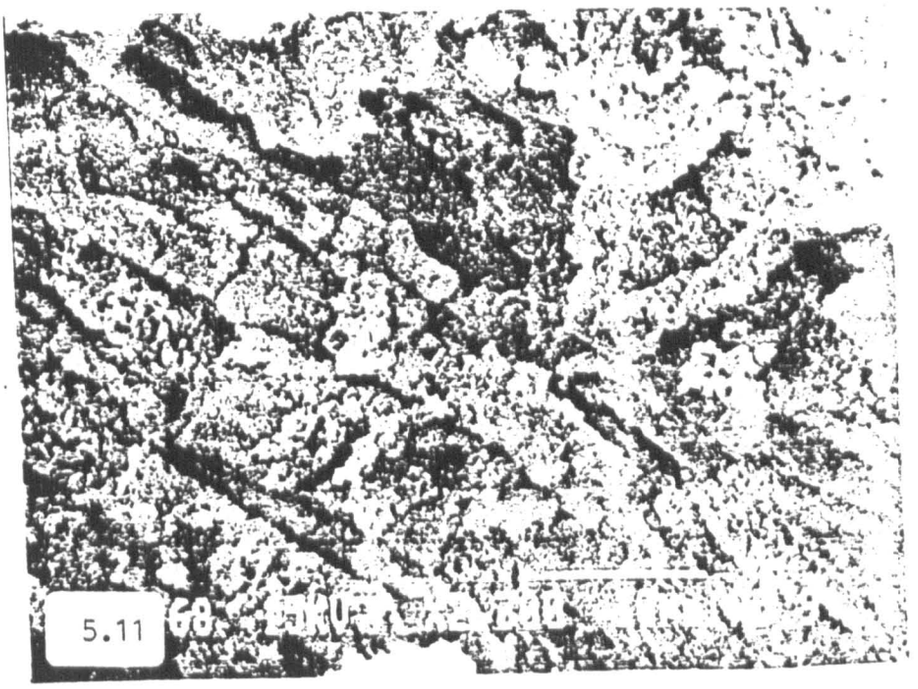
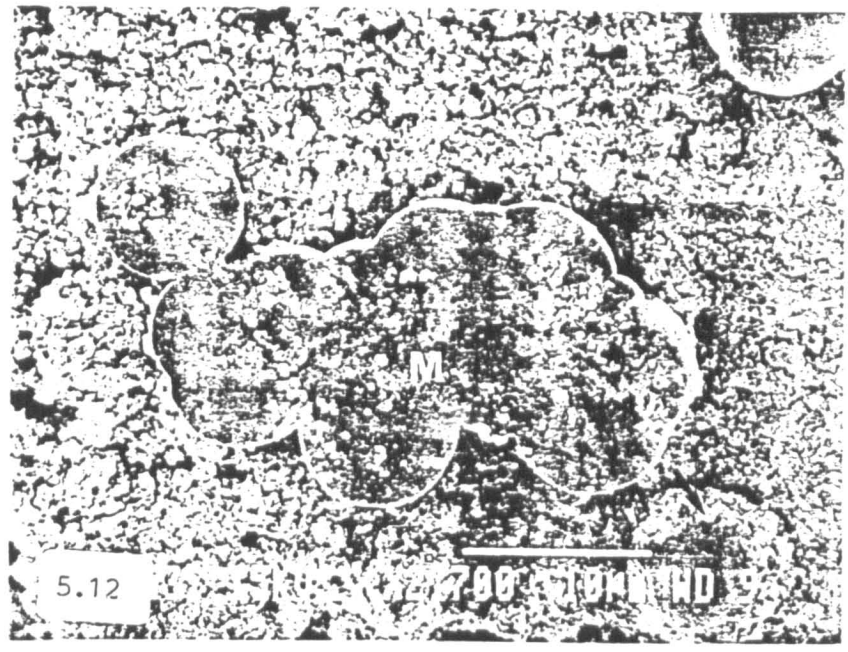
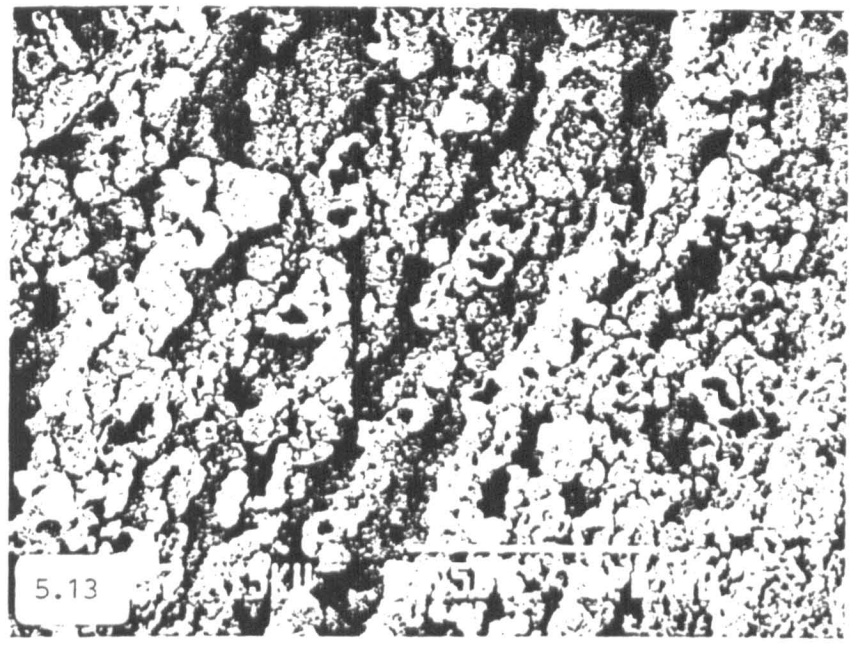

5.11

Figure 5.12: SEM. Muscle from the tentacles of *Plesioteuthis prisca* from the Solnhofen Limestone (BMNH C46871). The muscle is replaced by inorganic microspheres. The microbes ('M') are Recent contaminants. One (arrowed) has grown displacively in the intersticies of the phosphatized soft tissue. stub 91.

Figure 5.13: SEM. Muscle fibres (running NE-SW) from the tentacles of *Belemnotheutis antiquus* from the Lower Oxford Clay at Christian Malford (BMNH C46898). The fibres are crudely pseudomorphed by morphotype 4 microbes. Relative to morphotype 4 microbes infesting the soft tissues of the Romualdo Member biota, these microbes have a smoother external surface, are more frequently interconnected, and do not contain mineralized organelles. stub 89.

Figure 5.14: SEM. Terminal ends of muscle fibres (running NW-SE) from the tentacles of *Belemnotheutis antiquus* from the Lower Oxford Clay at Christian Malford (BMNH C46898). The fibres are preserved as external moulds by densely packed microbes. This has resulted in the fibres being preserved as a series of stacked hollow tubes. stub 89.


M
5.12

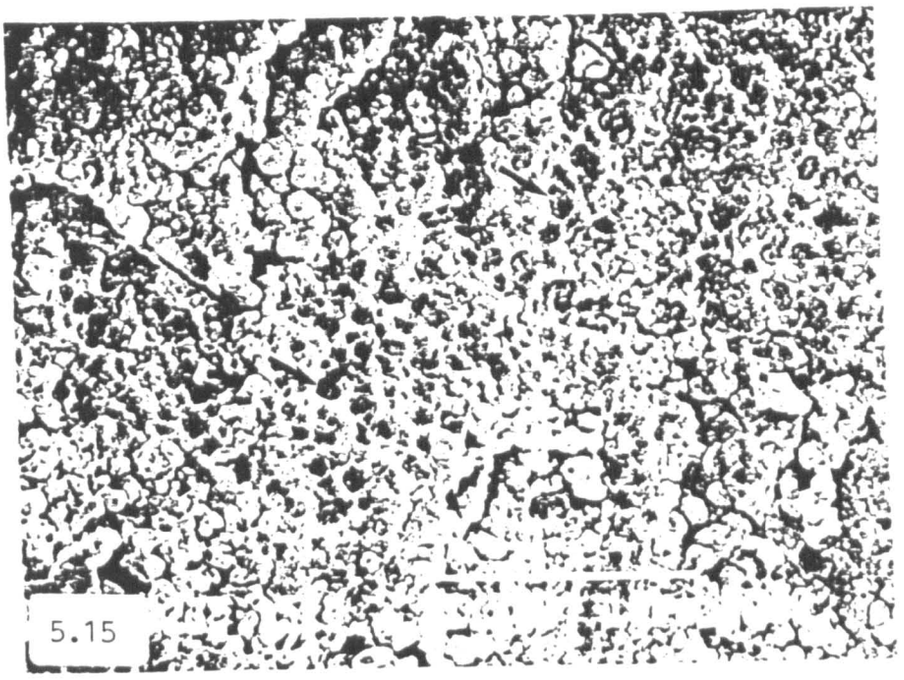

5.13


5.14

Figure 5.15: SEM. Phosphatized soft tissues of an ichthyosaur from the Lower Lias. The tissues are thoroughly decomposed, heavily infested with microbes, and smothered in 'microbial pits' (arrowed). The microbial pits represent the former position of microbes, and result from their metabolism of the substrate. stub 113.

Figure 5.16: SEM. Microbes pseudomorphing the soft tissues of an ichthyosaur from the Lower Lias. None of the tissue's original structure is preserved. The microbes are akin to morphotype 5 microbes but they are smaller, do not have spherical apertures, and have a more irregular shape. stub 112.

Figure 5.17: SEM. Muscle fibres (running NW-SE) from a *Tealliocaris* sp. from the Gullane shrimp bed. The fibres are pseudomorphed by morphotype 4 microbes. stub 138.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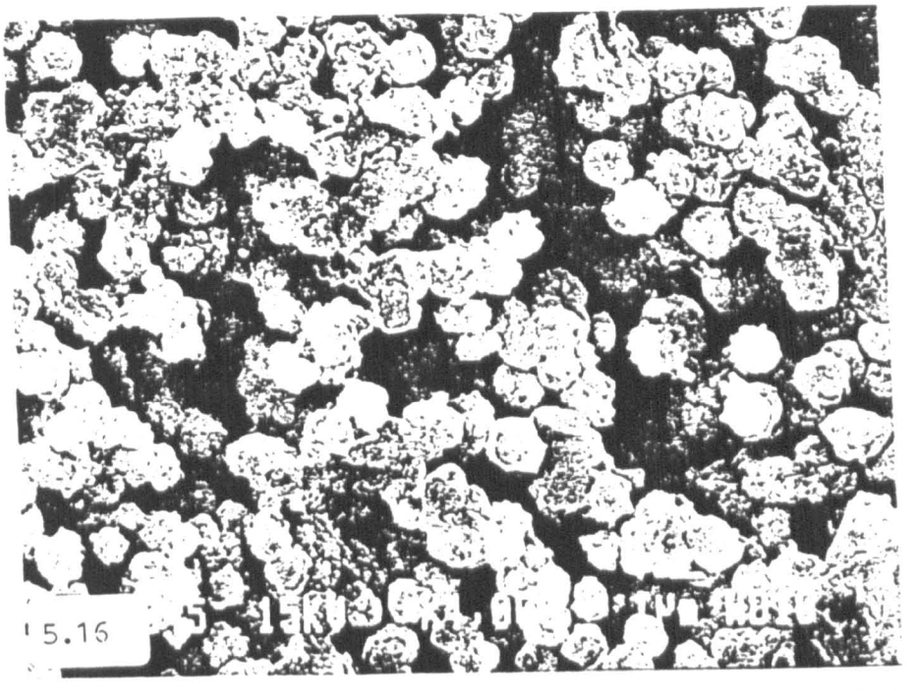
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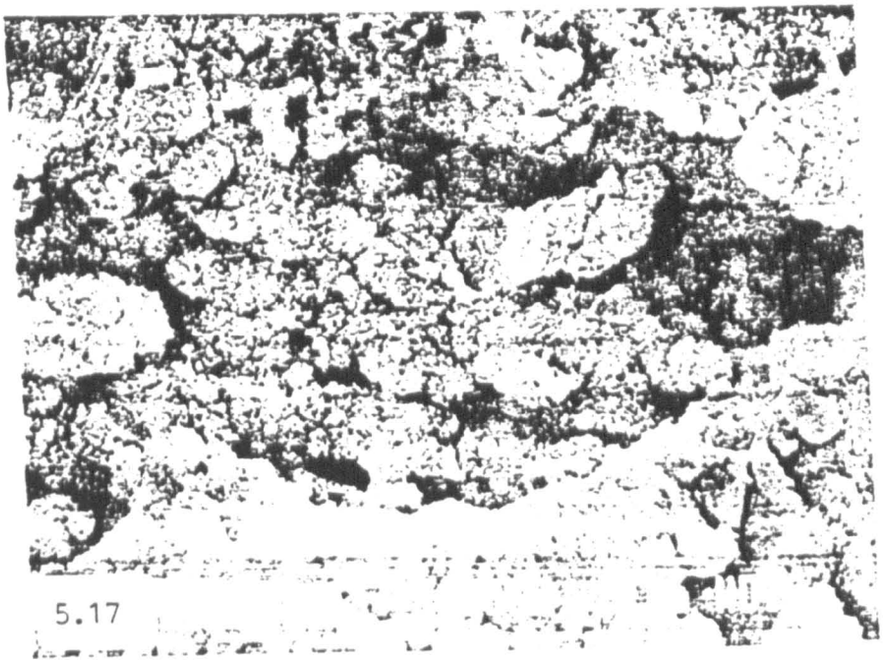
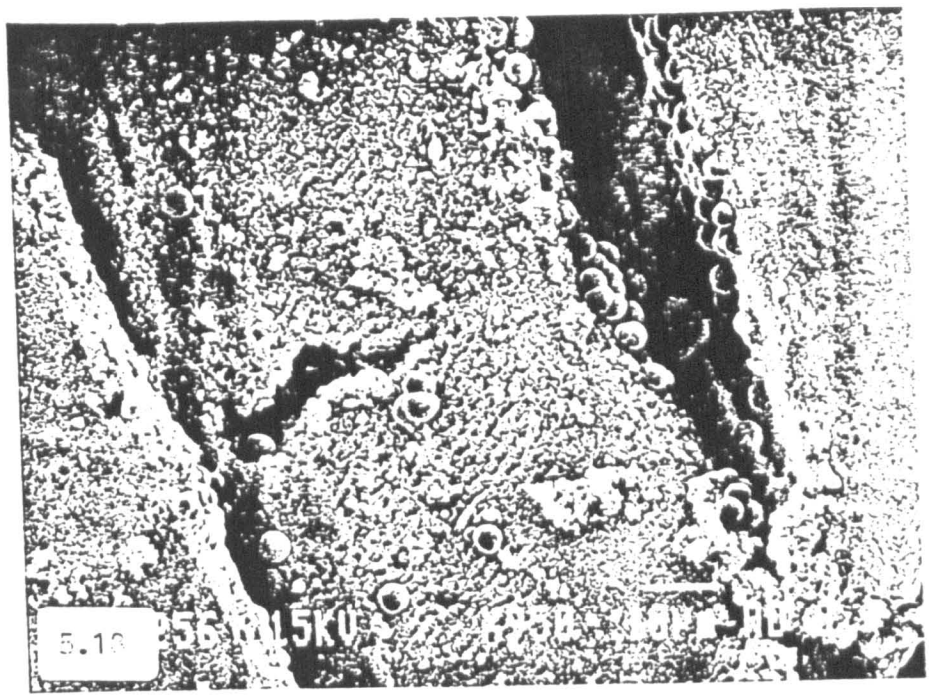

5.17

Figure 5.18: SEM. A striated muscle fibre replaced by microgranular apatite from *Ctenacanthus costellatus* of the Glencartholm Volcanic Beds (BMNH P5900). Transverse banding is well preserved. Morphotype 2 microbes form a prominent coating on some surfaces of the fibres. stub 13.

Figure 5.19: SEM. Three striated muscle fibres from a *Ctenacanthus costellatus* of the Glencartholm Volcanic Beds (BMNH P5900). The fibres are replaced by microgranular apatite and are coated by a single 'mat' of morphotype 2 microbes ('M'). The microbes are not in direct contact with the muscle fibres but are separated by a thin (<1μm) wavy gap (arrowed) which probably represents their sarcolemas. stub 223

Figure 6.1: SEM. Epithelial cells of the alimentary tract of a *Notelops* sp. The cells (some are outlined) cover the entire surface of the specimen and display considerable evidence of collapse and shrinkage. stub 135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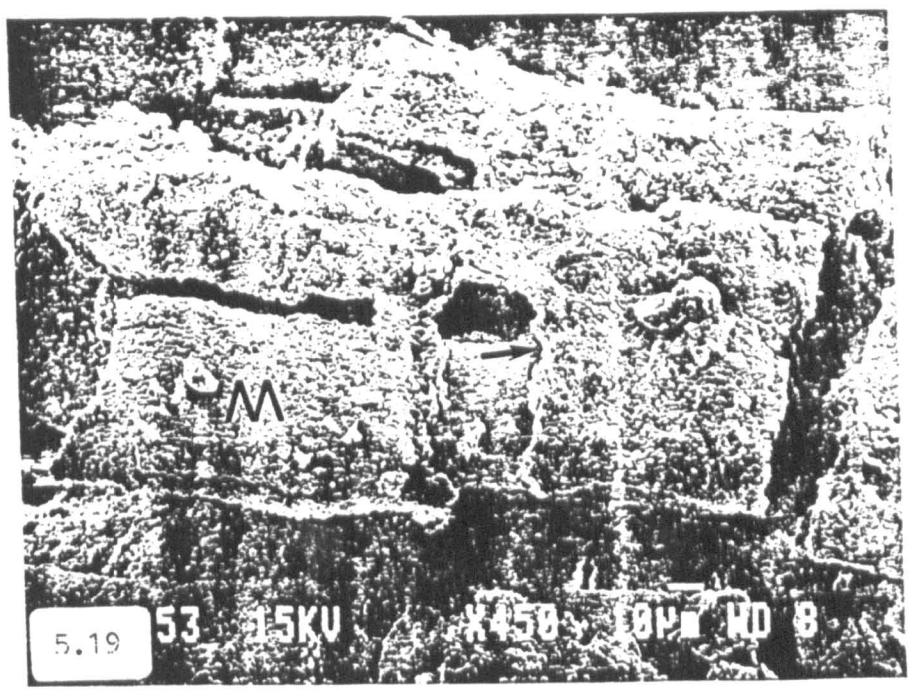

M
5.19
53 15KV X450 10µm WD 8

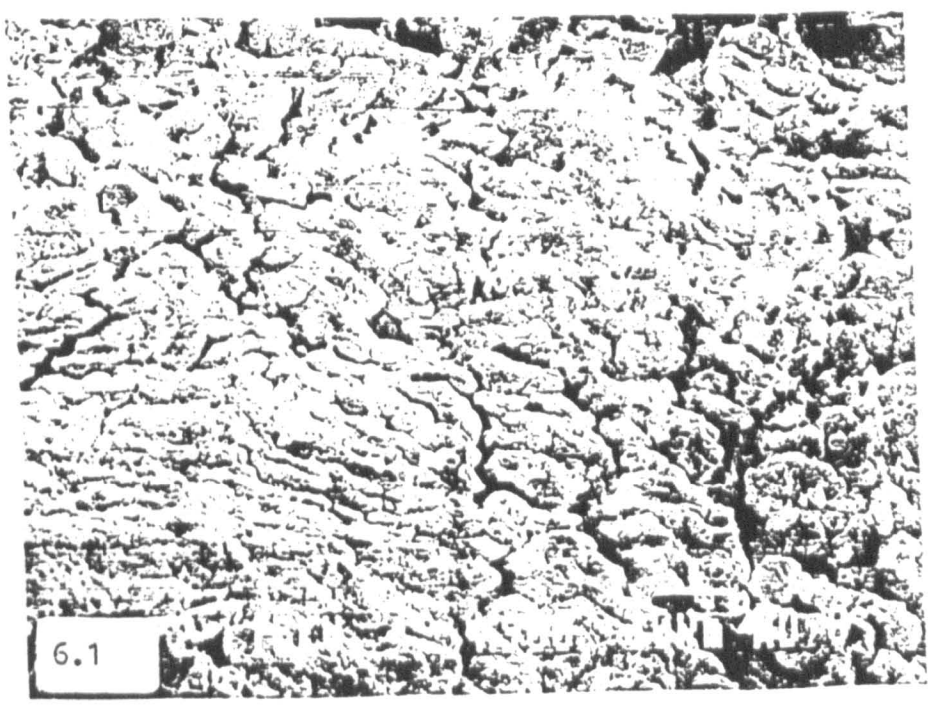

6.1

Figure 6.2: TEM. Two pristine cell nuclei on a striated muscle fibre ('M'). The nuclei are rounded, the nuclear envelopes ('E') taut, and the chromatin ('C') is concentrated along the inside of their inner membranes. The sarcolemma ('S') closely 'hugs' the nuclei. 1cm=1μm. Biological. Micro A1.

Figure 6.3: TEM. A decomposed striated muscle cell nucleus. The nuclear envelope is not taut and the nucleus has an irregular outline. The chromatin ('C') remains concentrated along the inside of the inner membrane. Collagen ('O') composing the sarcolemma is still perfectly banded. 1cm=0.66μm. Biological. Micro A3.

Figure 6.4: TEM. A decomposed striated muscle cell nucleus. The nucleus displays severe divergence from its original globose form. The chromatin is evenly dispersed within the nucleus, and the outer membrane ('O') has separated from the inner one ('I'). 1cm=0.5μm. Biological. Micro A5.

Figure 6.5: TEM. A decomposed striated muscle cell nucleus. The nucleus' outline is entirely irregular and its two membranes have parted around its entire circumference. Much of the chromatin has decomposed to leave empty spaces in the nuclear lumen (arrowed). The nucleus is still closely associated with the muscle fibre ('M'), but the sarcolemma is no longer present. 1cm=1.25μm. Biological. Micro A7.

Figure 6.6: TEM. A decomposed striated muscle cell nucleus. The nuclear envelope has been breached (arrowed) and the remaining chromatin ('C') is dispersed. Some portions of the nuclear envelope remain intact but the two membranes are widely separated ('E'). The muscle fibre ('M') is entirely structureless. 1cm=0.5μm. Biological. Micro A9.

6.4
6.3
6.2
6.6
6.5
M
C
e
i
o
s

Figure 6.7: TEM. A pristine mitochondrion. The organelle is globose with a taught outer membrane ('O'). The inner membrane is folded into many clearly identifiable cristae ('C'). 1cm=0.25µm. Biological. Micro B2.

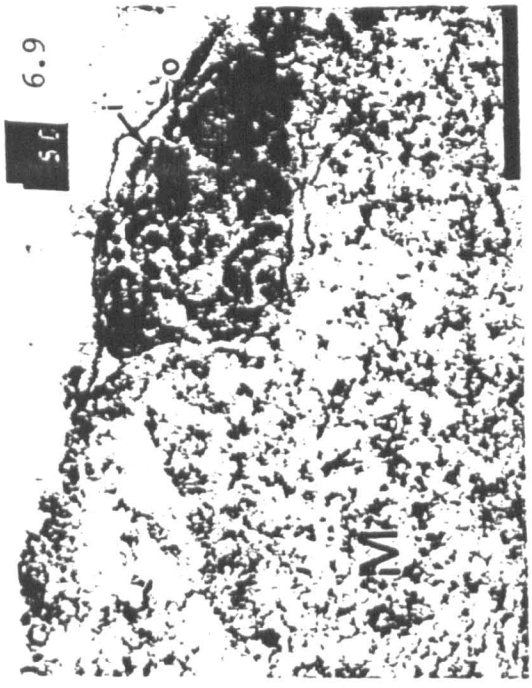
Figure 6.8: TEM. A decomposed mitochondrion in striated muscle ('M'). The two membranes have separated slightly in several places thereby enlarging the intermembrane space ('I'). The number of cristae ('C') is greatly reduced. The organelle as a whole retains its globular morphology. Biological. 1cm=0.3µm. Micro B4.

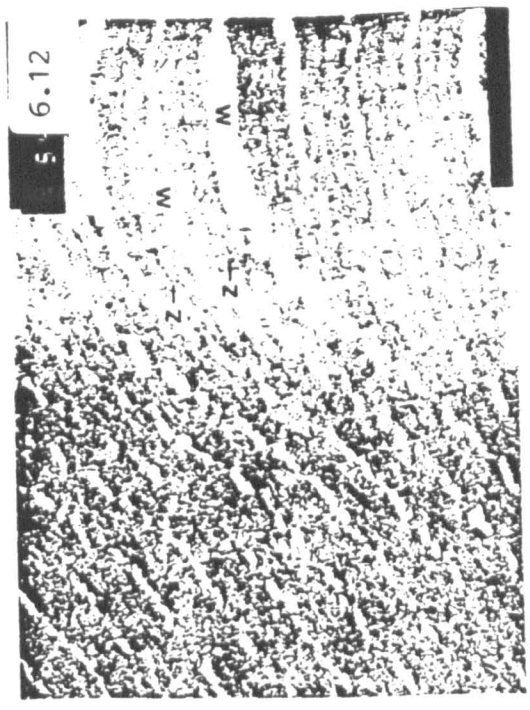
Figure 6.9: TEM. A decomposed mitochondrion. The organelle is 'deflated' and extremely irregular in outline. The inner- ('I') and outer-membranes ('O') have separated, and the cristae gave become indistinct and reduced in numbers. The muscle ('M') has become structureless. 1cm=0.2µm. Biological. Micro B6.

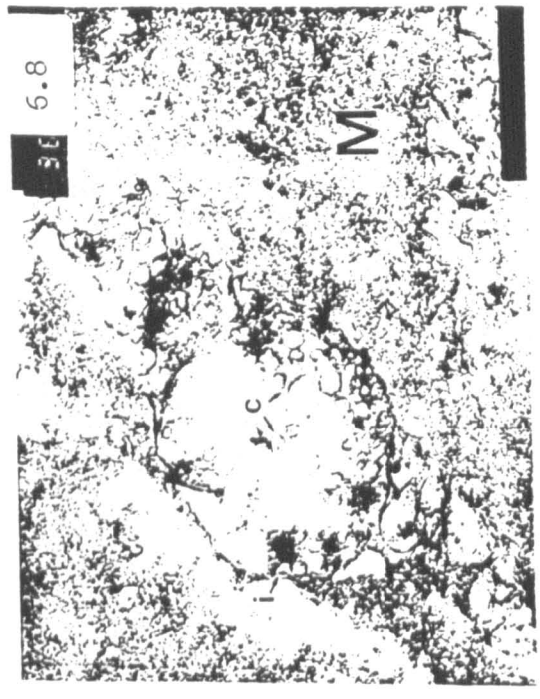
Figure 6.10: TEM. Pristine striated muscle myofibrils (running NW-SE). The Z-discs ('Z') of adjacent myofibrils are aligned parallel to one another, as are the M-lines ('M'). Each sarcomere ('S') is defined by two Z-discs. Individual actin- ('Ac') and myosin-fibrils ('My') can clearly be identified in the I- ('I') and A-bands ('A') respectively. The system of T-tubules and sarcoplasmic reticulum ('T') are the spherical bodies of low optical density adjacent to the Z-discs of each sarcomere. 1cm=0.66µm. Biological. Micro B2.

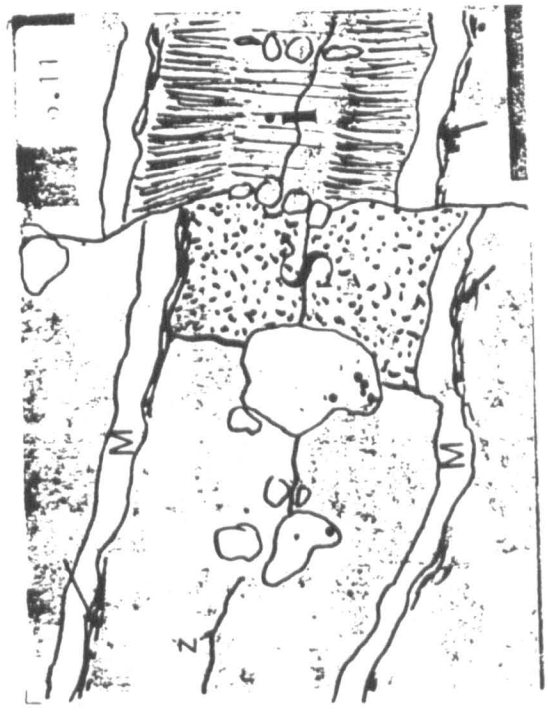
Figure 6.11: TEM. Striated muscle myofibrils (running N-S) disrupted by rigor mortis. Every sarcomere ('S') has contracted to such an extent that they have ruptured along their M-lines ('M'). The actin filaments remain firmly anchored to the Z-discs ('Z'). The occurrence of dark bands on only one side of the ruptured M-lines (arrowed) of *some* sarcomeres, suggests the myosin filaments in these sarcomeres to have been ripped free of the actin filaments on the other side of the rupture. Contraction of the sarcomeres has resulted in a shortening of the width of the I-bands ('I') relative to relaxed muscle. 1cm=0.5µm. Biological. Micro A7

Figure 6.12: TEM. Decayed striated muscle myofibrils (running N-S). Continued contraction and collapse of the fibre results in the ruptured M-lines ('M') enlarging in width and becoming irregular. The muscle fibre then consists of a series of laterally connected Z-discs ('Z') each forming a 'wall' in which the actin filaments of two halves of adjacent sarcomeres are firmly anchored. 1cm=2µm. Biological. Micro B8.


6.9
M


6.12
M
Z


6.8
M


M
M
Z


6.7

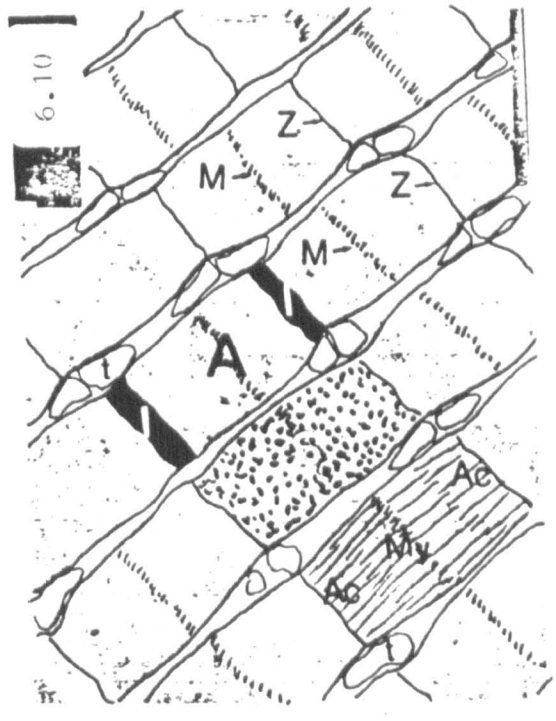

6.10
Z
M
Z
M
A
AC
M
AC

Figure 6.13: TEM. Decayed striated muscle myofibrils (running NE-SW). The myofibrils degrade into a series of loosely associated 'blocks', each consisting of a Z-disc ('Z') and two half sarcomeres. Some myosin filaments (arrowed) still straddle the enlarged and rather irregular M-lines, and the actin filaments ('A') in the blocks retain an ordered arrangement. The system of T-tubules and sarcoplasmic reticulum ('T'), although displaced, remains relatively intact. 1cm=0.66μm. Biological. Micro A7.

Figure 6.14: TEM. Decayed striated muscle myofibrils (running E-W). The actino-myosin bonds are damaged and the sarcomeres ('R') have adopted a stretched morphology with wide I-bands ('I'). Thin ruptures have developed along the M-lines ('M'). Although the actin and myosin filaments are in contact only along their terminal ends (and are therefore only loosely associated with one another), they are well ordered. The actin filaments are firmly anchored to the Z-discs ('Z'). The system of T-tubules and sarcoplasmic reticulum ('T') is well preserved. 1cm=0.5μm. Biological. Micro C1.

Figure 6.15: TEM. Decayed striated muscle myofibrils (running N-S). The width of the ruptured M-lines ('M') has increased and the myosin filaments have been pulled free of the actin filaments. The actin filaments ('A') remain attached to the Z-discs and lie flaccid in clusters between the disorganised blocks of myosin filaments ('B'). 1cm=0.66μm. Biological. Micro B10.

Figure 6.16: TEM. Complex taphonomic banding in striated fish muscle (myofibrils running NW-SE). The sarcomeres ('B') have ruptured along their M-lines ('M'), and the I-bands ('I') are severely stretched; the Z-discs ('Z') however remain intact. The bands consist of largely homogeneous blocks of severed myosin filaments ('H'), ruptured M-lines, stretched I-bands, and Z-discs. 1cm=1μm. Biological. Micro destroyed.

Figure 6.17: TEM. Decomposed striated muscle myofibrils (running NW-SE) replaced by microgranular apatite from a *Notelops* sp. As in figure 6.13, the M-line ('M') of each sarcomere ('S') is ruptured. Each mineralized block consists of the I-bands ('I') of two adjacent sarcomeres and an un-mineralized Z-disc ('Z'). Micro C3.

6.15
m
B
m
B
m

Z
M
I
I

R
Z
M
Z
M

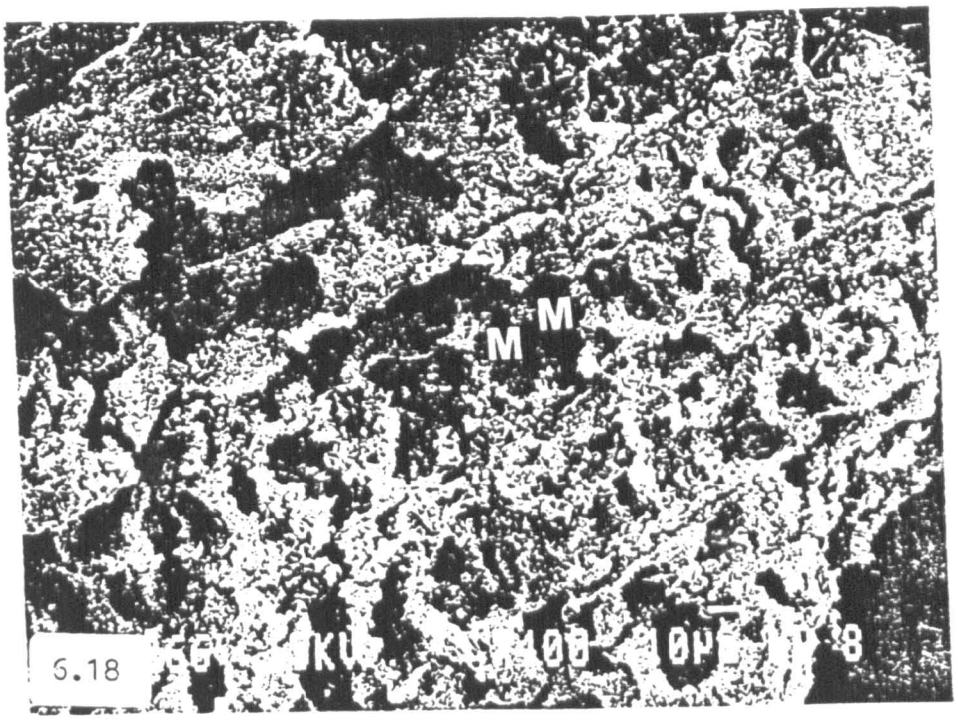
B
H
N
N

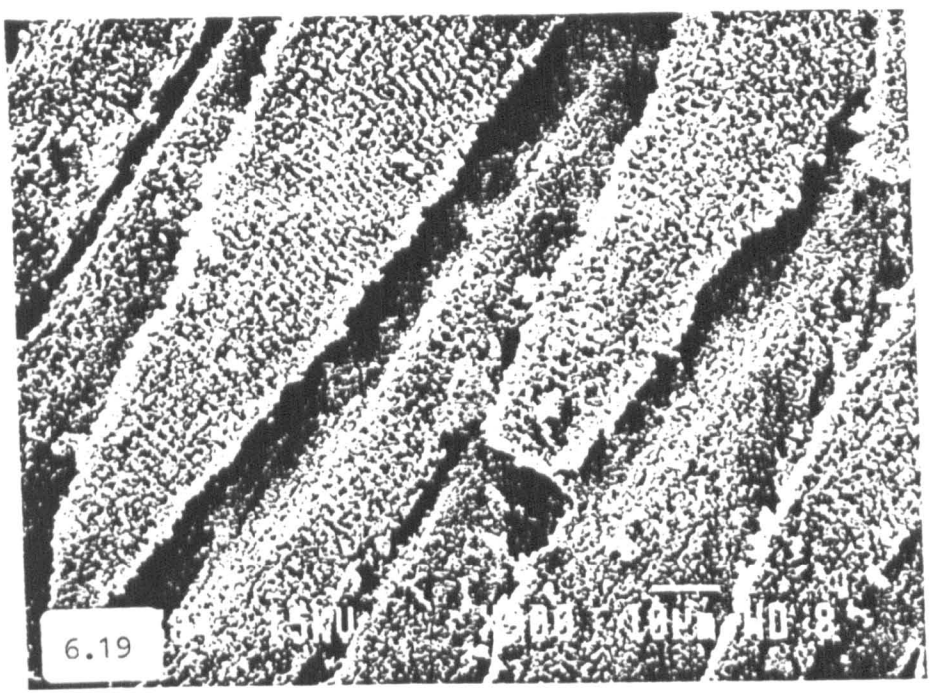
6.13
Z
Z

Figure 6.18: SEM. Decomposed striated muscle fibres (running NE-SW) from a *Rhacolepis* sp. The fibres have collapsed across a number of severely ruptured M-lines ('M'). stub 51.

Figure 6.19: SEM. Striated muscle fibres from a *Rhacolepis* sp. Relatively wide un-mineralized bands occur between thinner mineralized bands. The repeat sequence and width of bands in these fibres suggests that the mineralized bands correspond to clusters of flaccid actin filaments, and the un-mineralized bands represent disorganised blocks of myosin. This suggests phosphatization to be restricted almost entirely to the actin filaments. stub 79.

Figure 6.20: SEM. A single striated muscle fibre (running NE-SW) with a severley ruptured sarcolema preserved as an internal mould by inorganic microspheres. The sarcolema is torn in two places ('T') and its surface is contorted (arrowed) by longitudinal tensional stresses. stub 103


M
M
6.18

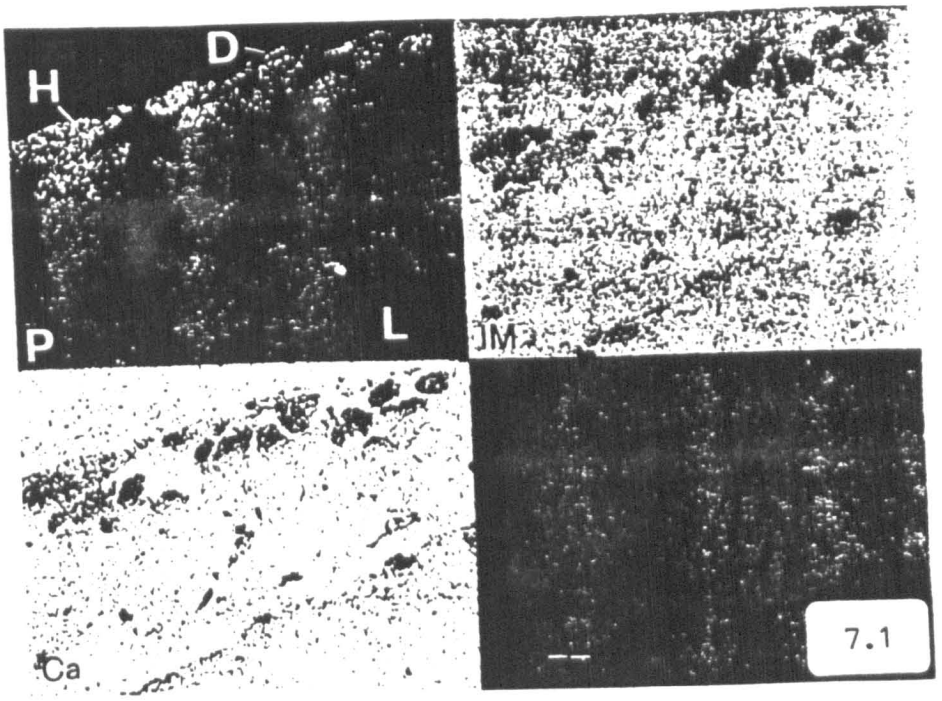

6.19

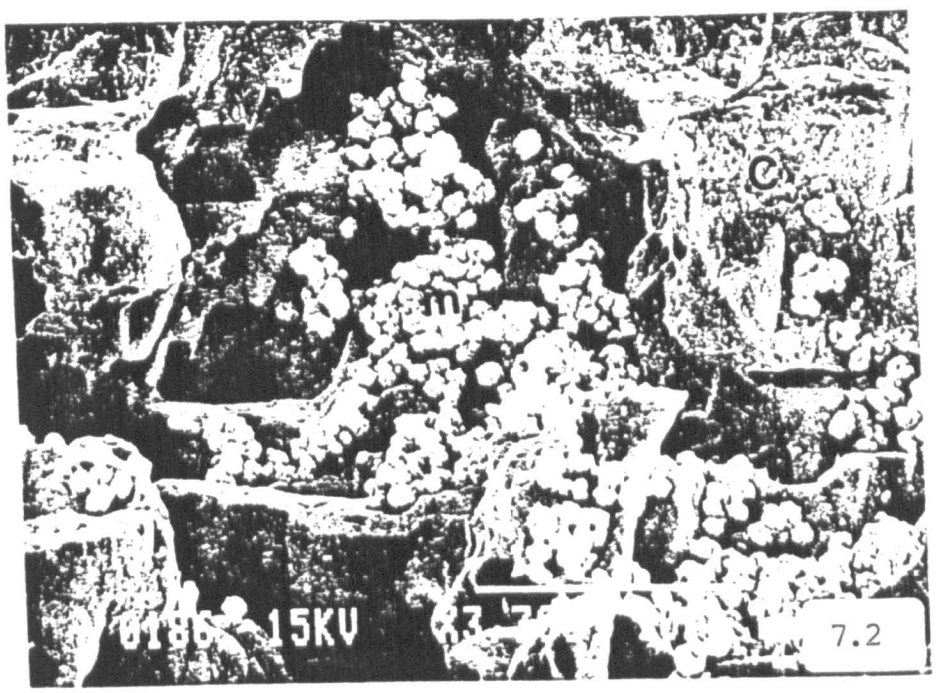

6.20

Figure 7.1: SEM (EDAX elemental maps). Phosphorus ('P') and calcium ('Ca') elemental maps, and backscattered image ('IM') of the peripheral white striated muscle fibres and dermis ('D') of a *Notelops* sp. The cross-sectional profile of individual muscle fibres are clearly visible. There is a gradational change in the density of phosphatization from a high at the periphery ('H'), to a low deeper within the fish ('L'). Slide C.

Figure 7.2: SEM. The density of mineralization of soft tissues a few millimetres beneath the dermis of a pterosaur. Inorganic microspheres ('M') do not preserve any of the tissue's ultrastructure and are largely isolated from one another. Large diagenetic calcites ('C') fill the interstitial spaces between the microspheres. Acid digestion of this specimen would result in its complete disintegration. stub is part of DNPM D6M 488 LE.

Figure 7.3: SEM. Two striated muscle fibres from the wing membrane of a pterosaur. The central myofibrils of one of the fibres are not preserved. This suggests a gradient in the density of mineralization to have existed in this fibre from a high just beneath its sarcolema, to a low at its centre. Banding is well preserved in the other fibre. stub is part of DNPM D6M 488 LE.


D
H
P
L
JM
Ca
7.1


C
m
15KV
7.2

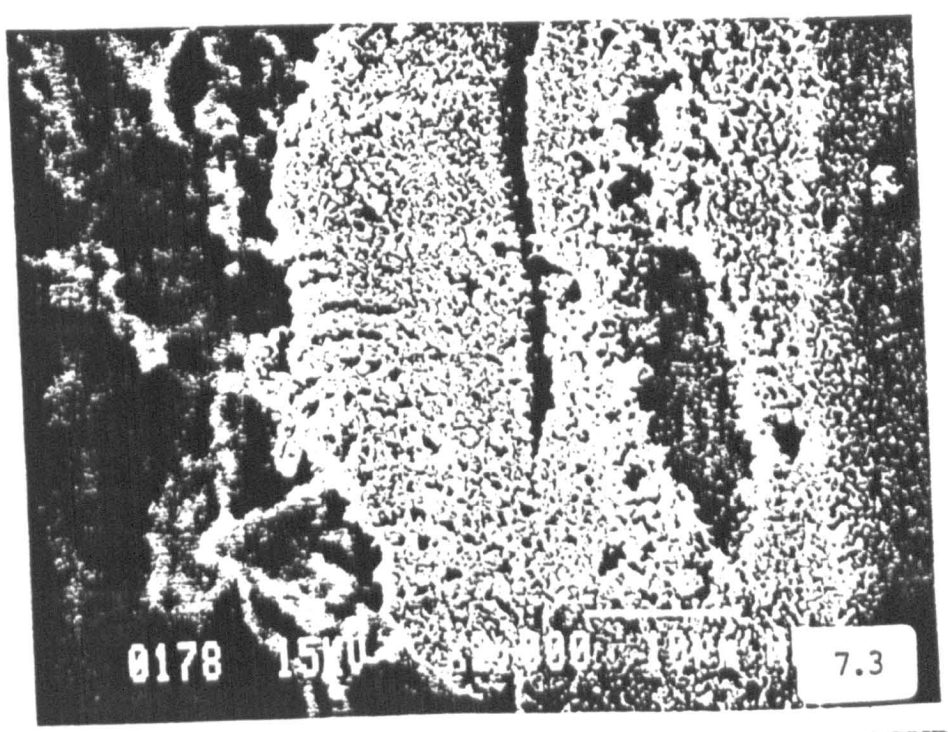
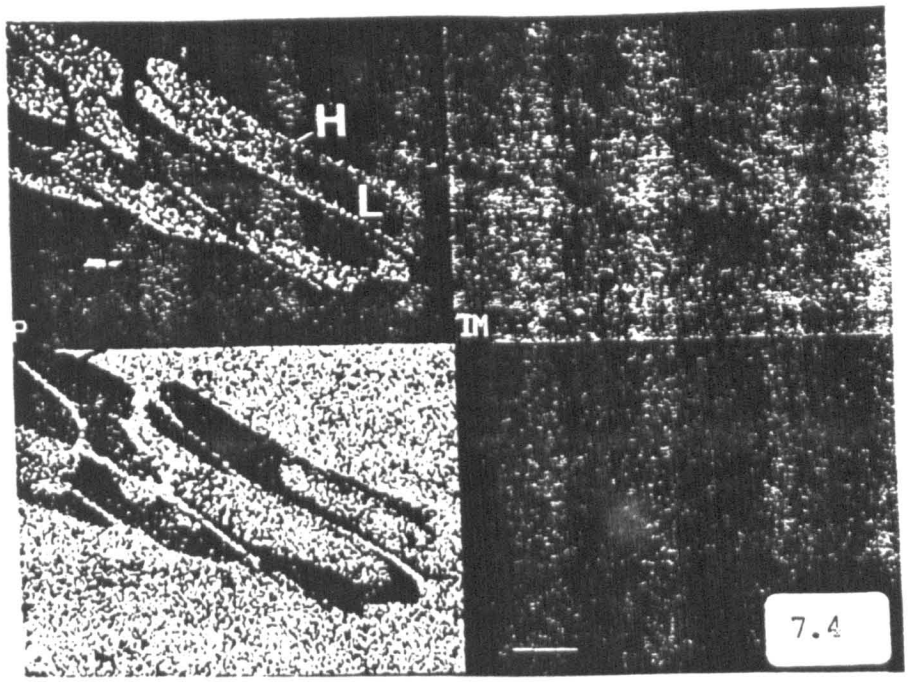
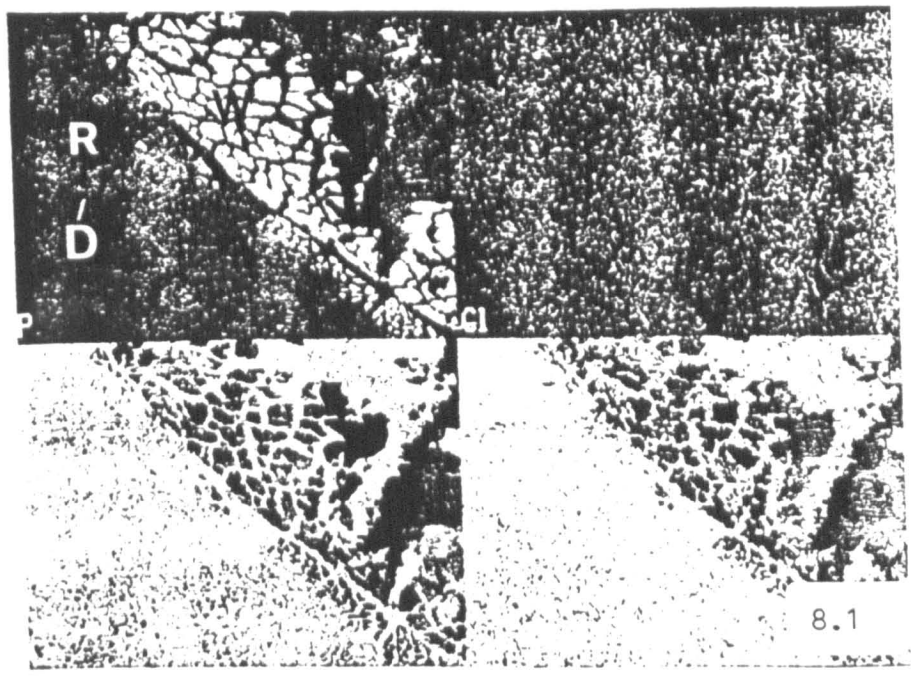

0178
7.3

Figure 7.4: SEM (EDAX elemental maps). Phosphorus ('P') and calcium ('Ca') elemental maps, and backscattered image ('IM') of several longitudinally sectioned striated muscle fibres from a *Rhacolepis* sp. There exists a clear their peripheries just beneath their sarcolemas, to a low ('L') at their centres. Slide D.

Figure 8.1: SEM (EDAX elemental maps). Phosphorus ('P'), chlorine ("Cl'), and calcium ('Ca') elemental maps, and backscattered image ('IM') of the peripheral red- ('R') and white-striated muscle fibres ('W') of a *Notelops* sp. The most densely phosphatized soft tissues are those ≈300μm beneath the dermis ('D') of the fish. The density of phosphatization decreases both above and below this zone. Slide E

Figure 8.2: SEM (EDAX elemental maps). Phosphorus ('P'), and calcium ('Ca') elemental maps, and backscattered image ('IM') of some transversely sectioned striated muscle fibres of a *Notelops* sp. Marked differences in the density of phosphatization of the muscle fibres occur on either side of the myoseptum ('M'). Fibres external to the myoseptum (i.e. those located towards the periphery of the carcass) are densely phosphatized ('H'), whereas those muscle fibres located internal of the myoseptum (i.e. in a position such that any phosphorus diffusing into the carcass from the external environment must diffuse across the myoseptum) are only very 'lightly' phosphatized ('L'). Slide F.


H
L
TM
7.4


R
D
8.1

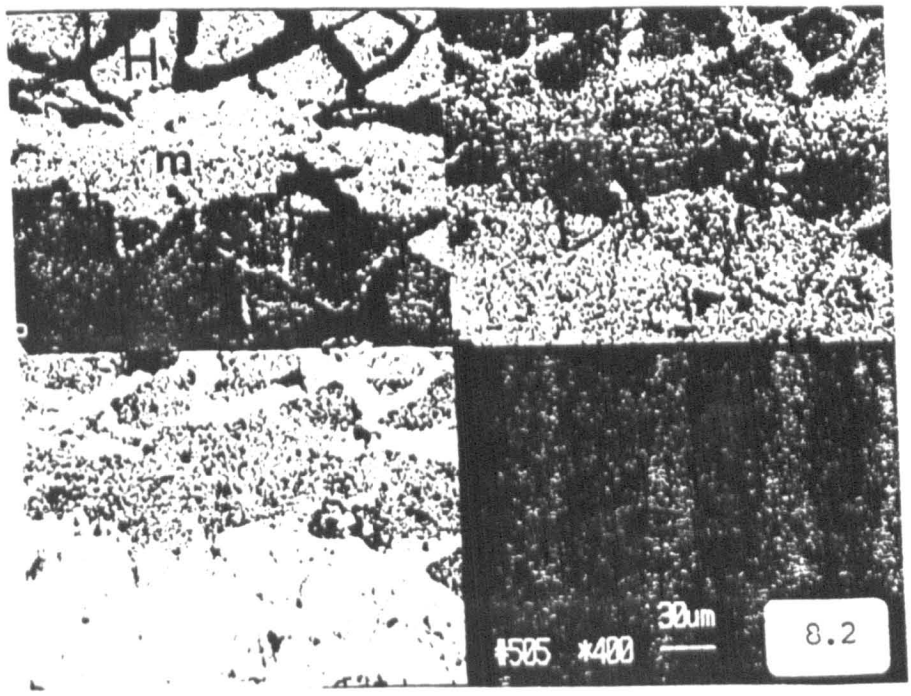
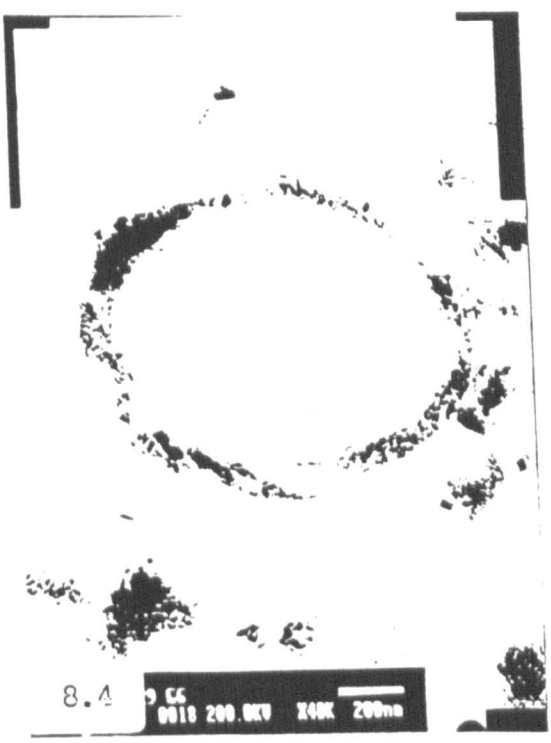

H
m
30um
#505 *400
8.2

Figure 8.3: TEM. Phosphatized cell from the alimentary tract of a *Rhacolepis* sp. The cell's organelles ('O') including the nucleus ('N') are preserved as internal moulds by microgranular apatite, and its plasma membrane ('P') is preserved by microgranular apatite as an external coating. The cytosol and endoplasmic reticulum ('C') is entirely un-mineralized. This implies three chemically independent compartments to have existed during mineralization: the organelles, the extracellular space, and the cytosol. Micro F2.

Figure 8.4: TEM. A phosphatized mitochondrion from the striated muscle of a *Rhacolepis* sp. Only the intermembrane space is phosphatized; the matrix, cristae, and external surface of the outer membrane were not involved in mineralization. Since the inner- and outer-membranes of mitochondria perform very different roles, they are likely to have very different compositions. Therefore, the selective mineralization of only the outer face of the inner membrane, and the inner face of the outer membrane, is unlikely to be substrate-driven. Instead, since the polarity of the two juxtaposed faces are the same, phosphatization was probably electrostatically-driven. Micro F4.


C
O
C
P
8.3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